Pharm

THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society for Analytical Chemistry

Editor: J. B. ATTRILL, M.A., F.R.I.C.
14 BELGRAVE SQUARE, LONDON, S.W.1
Telephone: BELgravia 3258
Published for the Society by
W. HEFFER & SONS LTD., CAMBRIDGE, ENGLAND

Volume 83 Price 10s. 6d.
Subscription Rate, inclusive of *Analytical Abstracts*, £6 6s. per annum, Post Free
No. 993, Pages 653-708 December, 1958

For revised prices from
January 1st, 1959, see overleaf

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society and the Association of Public Analysts was held at 3 p.m. on Wednesday, December 3rd, 1958, in the Wellcome Building, Euston Road, London, N.W.1. The meeting took the form of a Symposium on "Food Analysis."

During the afternoon session, the Chair was taken by the President of the Society, Dr. J. H. Hamence, M.Sc., F.R.I.C., and the following papers were presented and discussed: "The Determination of Chemical Antioxidants in Fats after Separation by Partition Chromatography," by K. G. Berger, M.A., N. D. Sylvester, M.Sc., F.R.I.C., and Miss D. M. Haines, B.Sc.; "The Estimation of Egg in Certain Foods by Enzymic Hydrolysis of the Phospholipids," by C. B. Casson, B.Sc., F.R.I.C., and F. J. Griffin, B.Sc., A.R.I.C.

Mr. H. E. Monk, B.Sc., F.R.I.C., P.A.I.W.E., President of the Association of Public Analysts, took the Chair at the evening session, which was opened by J. R. Nicholls, C.B.E., D.Sc., F.R.I.C. Under the general heading "The Identification of Coal Tar Colouring Matters in Foodstuffs," two papers were presented by P. S. Hall, B.Sc., F.R.I.C., and R. C. Spalding, M.A., F.R.I.C., and were discussed.

DEATHS

WE record with regret the deaths of

Alexander Hutcheon Bennett

William Lincolne Sutton.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Wednesday, October 15th, 1958, at the Kenilworth Hotel, 5 Queen Street, Glasgow, C.1. The Chair was taken by the Vice-Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following papers were presented and discussed: "The Determination of Acidity in Dark Lubricating Oils," by W. Gibb, B.Sc., Ph.D., A.R.I.C., A.R.C.S.T., A.M.Inst.F., and H. Gibson, B.Sc. (see summary below); "Volumetric Analysis of Stannous and Total Tin in Acid-soluble Tin Compounds," by J. D. Donaldson, B.Sc., and W. Moser, B.Sc., A.R.I.C.

THE DETERMINATION OF ACIDITY IN DARK LUBRICATING OILS

DR. W. GIBB said that the deterioration of mineral lubricating oils under conditions found in internal combustion engines was primarily the result of the reaction of oxygen with the oil at elevated temperatures. Acidic bodies, *inter alia*, were formed, and the oil became dark coloured. The determination of acid number was complicated by this dark colour, the weak acidity and the small amounts present. The direct determination of the oxygen content of the deteriorated oil by the Unterzaucher method (*Analyst*, 1952, **77**, 584) now made possible an oxygen balance and the separate determination of the various groups of acidic constituents of major importance.

Two-phase colour indicator methods of acid-number determination were unreliable (H. P. Ferguson, *Anal. Chem.*, 1950, **22**, 289) owing to the absorption of carbon dioxide, saponification and emulsion formation. With heavily oxidised oils of high acid number,

even I.P. 1/55 method A ("Standard Methods for Testing Petroleum and Its Products," The Institute of Petroleum, London, 1957, p. 13) could give a two-phase system. The newer method I.P. 139/57T was only suited to lightly oxidised material, as the solvent (*iso*propanol - benzene - water) had difficulty in dissolving heavily oxidised sludges, and the *p*-naphtholbenzein end-point was difficult to see in black solutions. Determination of the end-point was improved by observing the colour change in froth (*Ibid.*, p. 4), in narrow-bore glass tubing (*Ibid.*, p. 10), in special titration flasks with narrow necks or narrow side-limbs (*Ibid.*, p. 13; I. Kukin, *Anal. Chem.*, **1957**, **29**, 461) or by means of fluorescent lamps ("Standard Methods for Testing Petroleum and Its Products," p. 4), or a photoelectric colorimeter (R. H. Osborne, J. H. Elliott and A. F. Martin, *Ind. Eng. Chem., Anal. Ed.*, **1943**, **15**, 642).

The A.S.T.M. potentiometric method D664 ("A.S.T.M. Standards," American Society for Testing Materials, Philadelphia, Pa., U.S.A., 1955, part 5, p. 285) made use of the same solvent as I.P. 139/57T. The technique was time-consuming, and the electrodes had to be scrupulously clean and carefully shielded from electrical interference. Since most oxidised samples gave no points of inflexion on the titration curve, end-points had to be taken at meter readings corresponding to suitable standard buffer solutions. Potentiometric titration of oxidised oils in a strongly basic solvent had attractive possibilities, since in such a medium weak acids showed a much greater acid strength than in water or alcohols (M. L. Moss, J. H. Elliott and R. T. Hall, *Anal. Chem.*, 1948, **20**, 784; V. Z. Deal and G. E. A. Wyld, *Ibid.*, 1955, **27**, 47). Accordingly, potentiometric curves that did not show points of inflexion in alcohol solvents should show them in a suitably basic solvent, so permitting differentiation between carboxylic acids and phenols. With basic solvents, such as ethylenediamine, a totally enclosed anhydrous titration system was necessary. Anomalous titration curves had been reported for insufficiently basic solvents (H. B. van der Heijde, *Anal. Chim. Acta*, 1957, **16**, 378).

For the rapid determination of the acid number of very dark oils, Fenske's fluorescein - methyl red indicator (*Ind. Eng. Chem.*, 1941, **13**, 51) had been used successfully. The red form of methyl red masks the fluorescence, which can be made to show up clearly on the alkaline side. Observation of the end-point is aided by illuminating the titration vessel strongly from the side and using a dark background to reduce transmitted light. No difficulty had been experienced in dissolving the sludges of heavily oxidised oils in the *n*-butanol - toluene solvent. The titrant was sodium butylate. Improved accuracy could be obtained with insufficiently dark samples by the addition of a neutral black dye to the oil solution.

A complete oxygen balance also required the determination of carbonyl compounds. The oil was treated with hydroxylamine hydrochloride and the liberated acid was determined potentiometrically (J. Knotnerus, *J. Inst. Petrol*, 1956, **42**, 355). A.S.T.M. method D664 was applicable. For rapid determination of liberated acid, the Fenske method in its original form was useless, as sodium butylate and methyl orange react with hydroxylamine hydrochloride. I.P. 139/57T solvent and titrant in conjunction with fluorescein - methyl orange indicator had been used successfully.

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, October 31st, 1958, in the Lecture Room of the Royal Society of Edinburgh, 22 George Street, Edinburgh 2. The Chair was taken by the Chairman of the Section, Dr. Magnus Pyke, F.R.I.C., F.R.S.E.

The following paper was presented and discussed: "The Analytical Chemistry of Phosphorus," by N. T. Wilkinson, F.R.I.C.

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Wednesday, November 19th, 1958, in the Upper Hall of the Royal Philosophical Society of Glasgow, 207 Bath Street, Glasgow, C.2. The Chair was taken by the Chairman of the Section, Dr. Magnus Pyke, F.R.I.C., F.R.S.E.

The subject of "Developments in Gas Chromatography" was introduced by A. F. Williams, B.Sc., F.R.I.C., and the following papers were presented and discussed: "Quantitative Analysis Using Thermal Conductivity Detection," by G. R. Jamieson, B.Sc., F.R.I.C.; "The Application of Gas Chromatography to Reaction Kinetics," by J. H. Knox, B.Sc., Ph.D.; "Chromatographic Examination of a Low-temperature Tar," by L. Irvine, B.Sc., Ph.D., A.R.C.S.T., A.R.I.C.

MIDLANDS SECTION

A JOINT Meeting of the Midlands Section and the Birmingham and Midlands Section of the Royal Institute of Chemistry was held at 7 p.m. on Thursday, November 13th, 1958, in the Main Chemistry Theatre, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Midlands Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

The following paper was presented and discussed: "The Infra-red Analysis of Solid Substances," by Professor G. Duyckaerts (Liège University).

Determination of Zinc and Other Elements in Plants by Atomic-absorption Spectroscopy

BY D. J. DAVID

(Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., Australia)

Results of investigations into the application of atomic-absorption spectroscopy to the analysis of plant material for zinc, magnesium, copper and iron are given. For zinc and magnesium, the method is at least as accurate and sensitive as other methods currently available, and is considerably better in both rapidity and freedom from interference by extraneous elements. For copper and iron, the method is insufficiently sensitive for general application in its present form.

THE aim of the work described was to test the application of atomic-absorption spectroscopy, developed by Walsh[1] and Russell, Shelton and Walsh,[2] to the analysis of plant material for certain inorganic elements, particularly zinc.

Most methods currently available for determining zinc in plant material involve preliminary chemical concentration with a solution of dithizone or another complexing reagent in chloroform or carbon tetrachloride. The dithizone extract can be subjected to arc-emission spectrographic analysis,[3] to further extraction to remove interfering elements and then photometric measurement of the zinc - dithizone complex[4] or to evaporation, digestion and polarographic analysis.[5] Atomic-absorption spectroscopy has advantages over these methods in that the diluted plant digest is used directly and special precautions to avoid interference from other ions of plant origin are not necessary.

Verdier, Steyn and Eve's polarographic method[6] and a method in which zinc is separated with Dowex 1 ion-exchange resin and then colorimetrically determined with Zincon[7] both avoid the use of complexing reagents in organic solvents. When compared with the proposed method, however, they have the disadvantage that some chemical preparation of the solution is necessary before the zinc can be determined, and the polarographic method, at least, is inferior in sensitivity and accuracy. Calculations based on the data reported for the polarographic method[6] suggest that the lower limit of determination of zinc in solution is about 1 p.p.m., but no estimate of accuracy is given at this level. At 31·5 p.p.m. of zinc in solution, a standard deviation of $\pm 1{\cdot}26$ p.p.m. is claimed. Six operations are necessary to prepare a solution for polarographic analysis by this method.

DESCRIPTION OF APPARATUS

A Lundegårdh air - acetylene flame of the type described by Mitchell,[8] into the base of which a fog of the sample solution is introduced, was placed on the optical axis between the slit of a flat-field Hilger medium-quartz spectrograph and a hollow-cathode discharge tube, which emitted intermittent light of the element to be determined at a frequency of 50 cycles per second. The plate holder of the spectrograph was replaced by a horizontal platform carrying a slit and photomultiplier assembly, which could be moved along guide grooves so that the slit remained in the focal plane of the spectrograph. During determinations, this exit slit was placed on the resonance line of the element to be determined and the transmitted light was picked up by an RCA 1P28 photomultiplier tube. The signal from the photomultiplier tube was fed to an a.c. amplifier tuned at 50 cycles per second, and the rectified output was measured with a millivoltmeter.

Earlier apparatus described by Russell, Shelton and Walsh[2] was similar in principle to that used in this investigation, but differed slightly in that a mechanical chopper was used to modulate the hollow-cathode beam and the signal from the a.c. amplifier was rectified and fed into a pen recorder.

The use of an intermittent hollow-cathode discharge and an a.c. amplifier precludes all interference by flame-emitted light.

As the apparatus described operates on the single-beam principle and the intensity of light emitted from the hollow-cathode discharge tubes is sensitive to slight fluctuations in mains voltage, an electronic a.c. voltage-stabiliser that delivered 240 ± 1 volts was used to supply the hollow-cathode tubes.

When the apparatus was used for analysis, the amplifier was adjusted to zero with the spectrograph-slit shutter closed and to full-scale deflection with the shutter open and a fog of pure water entering the base of the flame. The percentage reduction in reading when the water fog was replaced by a fog of sample solution was a measure of the absorption by the flame of the resonance line of the element to be determined.

Adjustment to full-scale deflection was effected by varying either the gain of the amplifier or the voltage applied to the photomultiplier stages.

The air and acetylene pressures applied to the Lundegårdh flame assembly, which were kept constant by means of reducing valves during determinations, were 36 lb per sq. inch and 40 cm of water, respectively. Other equipment settings used are shown in Table I.

TABLE I

INSTRUMENT SETTINGS FOR THE DETERMINATION OF ZINC, MAGNESIUM, COPPER AND IRON

Element	Width of entrance slit, mm	Width of exit slit, mm	Hollow-cathode tube current, mA	Wavelength of spectral line, A
Zinc	0·10	0·2	10	2139
Magnesium ..	0·15	0·2	10	2852
Copper	0·20	0·2	20	3247
Iron	0·05	0·2	50	3758

EXPERIMENTAL

REPRODUCIBILITY OF THE METHOD—

Atomic-absorption readings on thirty-nine portions of each of two zinc solutions in thirty-nine Lundegårdh spray bulbs gave results of 58·56 ± 1·07 per cent. absorption at a zinc level of 10 p.p.m. and 7·26 ± 0·94 per cent. absorption at a zinc level of 1 p.p.m. These variations, which are standard deviations of single determinations, may originate from variations in the dimensions of the spray bulbs, from electrical variation, from variations in acetylene and air pressures or from inaccuracies when readings are made.

A similar test for magnesium gave results of 10·37 ± 0·33 per cent. absorption at a magnesium level of 0·5 p.p.m. and 43·15 ± 1·01 per cent. absorption at a magnesium level of 5 p.p.m. The variation at the latter level includes a slight drift similar to that in the magnesium results shown in Table II.

TESTS FOR INTERFERENCES—

A solution containing all the water-soluble major elements likely to be encountered in plant material was prepared by dissolving 10 g of potassium chloride, 2 g of sodium chloride, 4 g of calcium carbonate, 1 g of magnesium oxide, 3 g each of ammonium dihydrogen orthophosphate and ammonium sulphate and 0·4 g of aluminium ammonium sulphate in sufficient hydrochloric acid to convert the calcium carbonate and magnesium oxide to chlorides. This solution was diluted to 1 litre with water, which gave a solution approximately equivalent to that obtained when 0·2 g of the ash from about 2 g of dry plant material (from an "average" pasture sample[8]) is dissolved in a volume of 10 ml. Atomic-absorption measurements at 2139 A were made for zinc at six concentration levels in this solution and in water; the results were as follows—

Amount of zinc present, p.p.m.	1	2	4	8	16	32
Absorption in water, %	10	18·5	34	59	86	100
Absorption in synthetic plant-ash solution, % ..	11	21	35	60	88	100

To study the interference of individual inorganic plant-elements on the absorption at four levels of zinc, copper and iron, each of the ions K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, SO_4^{2-} and PO_4^{3-} was varied individually between zero and from two to ten times its concentration in the solution mentioned in the previous paragraph, the concentrations of the other ions remaining

TABLE II

EFFECT OF MAJOR ELEMENTS IN PLANT MATERIAL ON THE ATOMIC ABSORPTION OF ZINC, IRON, COPPER AND MAGNESIUM

All concentrations, except those for magnesium, must be divided by 100 for application to magnesium

Possible interfering element	Amount of element present, %	Absorption of—				Absorption of—			
		0 p.p.m. of zinc, %	0 p.p.m. of iron, %	0 p.p.m. of copper, %	6 p.p.m. of magnesium, %	1 p.p.m. of zinc, %	20 p.p.m. of iron, %	2 p.p.m. of copper, %	6 p.p.m. of magnesium, %
Sodium ..	0·0	0·0	0·2	0·4	50·0	4·0	0·8	1·6	50·0
	0·078	0·0	0·0	0·0	52·0	4·0	0·8	1·6	50·0
	0·78	0·0	0·0	0·4	50·0	4·0	0·6	1·8	50·0
Potassium ..	0·0	0·0	0·0	0·0	48·0	4·0	0·8	1·8	50·0
	0·52	0·0	0·2	0·0	50·0	4·0	1·0	1·8	50·0
	1·04	0·0	0·0	0·0	48·0	4·0	1·0	1·8	50·0
Calcium ..	0·0	0·0	0·0	0·0	44·0	4·0	1·4	1·8	46·0
	0·16	0·0	0·0	0·0	48·0	4·0	1·2	1·8	46·0
	0·80	0·0	0·0	0·2	48·0	4·0	1·2	1·8	46·0
Magnesium	0·0	0·0	0·0	0·0	—	4·0	0·6	1·8	—
	0·06	0·0	0·0	0·0	—	4·0	0·4	2·0	—
	0·30	0·0	0·0	0·0	—	6·0	0·4	1·8	—
Phosphorus	0·0	0·0	0·0	0·0	46·0	6·0	0·6	1·6	46·0
	0·081	0·0	0·0	0·0	44·0	6·0	0·6	1·6	46·0
	0·403	0·0	0·2	0·0	44·0	6·0	0·4	1·6	44·0
Sulphur ..	0·0	0·0	0·0	0·0	42·0	4·0	0·4	2·0	42·0
	0·023	0·0	0·0	0·0	44·0	4·0	0·4	2·0	44·0
	0·115	0·0	0·0	0·2	42·0	4·0	0·6	1·4	44·0
Aluminium	0·0	0·0	0·0	0·0	44·0	4·0	1·2	1·2	44·0
	0·0024	0·0	0·0	0·0	42·0	4·0	1·2	1·2	44·0
	0·012	0·0	0·0	0·0	44·0	4·0	0·8	1·2	42·0

Possible interfering element	Amount of element present, %	Absorption of—				Absorption of—			
		4 p.p.m. of zinc, %	80 p.p.m. of iron, %	8 p.p.m. of copper, %	6 p.p.m. of magnesium, %	8 p.p.m. of zinc, %	160 p.p.m. of iron, %	16 p.p.m. of copper, %	6 p.p.m. of magnesium, %
Sodium ..	0·0	14·0	4·0	5·8	50·0	30·0	7·0	10·6	50·0
	0·078	14·0	4·0	6·0	50·0	30·0	7·0	10·6	50·0
	0·78	14·0	4·0	5·4	50·0	30·0	7·0	10·6	50·0
Potassium ..	0·0	14·0	4·2	5·8	48·0	30·0	7·0	10·4	48·0
	0·52	14·0	4·0	5·8	50·0	30·0	7·0	10·4	48·0
	1·04	14·0	4·0	5·8	48·0	30·0	7·0	10·6	46·0
Calcium ..	0·0	14·0	3·6	5·6	46·0	30·0	7·4	10·6	46·0
	0·16	14·0	4·0	5·8	48·0	30·0	6·4	10·4	48·0
	0·80	14·0	4·2	5·6	48·0	30·0	6·6	10·0	48·0
Magnesium	0·0	18·0	2·0	6·0	—	40·0	3·2	12·0	—
	0·06	18·0	1·4	6·2	—	36·0	3·4	12·0	—
	0·30	18·0	1·6	6·0	—	40·0	3·2	11·6	—
Phosphorus	0·0	18·0	1·8	6·0	46·0	44·0	3·8	12·2	46·0
	0·081	18·0	1·8	6·0	46·0	46·0	4·0	13·7	44·0
	0·403	16·0	1·8	6·2	44·0	48·0	3·6	14·0	44·0
Sulphur ..	0·0	16·0	1·8	6·0	44·0	44·0	4·0	12·0	44·0
	0·023	16·0	1·4	5·8	48·0	43·0	4·0	12·0	44·0
	0·115	14·0	1·4	4·6	44·0	45·0	3·6	11·7	42·0
Aluminium	0·0	12·0	3·6	4·6	44·0	24·0	6·0	8·2	44·0
	0·0024	12·0	2·8	4·4	42·0	24·0	6·0	8·2	42·0
	0·012	12·0	3·6	4·2	42·0	24·0	6·6	8·6	42·0

unchanged. The details of zinc, copper and iron levels and the results of the experiment are shown in Table II.

A similar study of the interference of K^+, Na^+, Ca^{2+}, Al^{3+}, SO_4^{2-} and PO_4^{3-} on magnesium absorption was made by measuring the magnesium absorption after hundredfold dilution of the solutions from the previous test. These results are also shown in Table II, but it must be borne in mind that all solution concentrations, except those heading the magnesium columns, should be divided by 100 for application to the magnesium results.

As a residual amount of sulphuric acid is present in a solution prepared by digesting plant material with a mixture of sulphuric, perchloric and nitric acids, the effect of high concentrations of sulphuric acid on the absorption of zinc, copper, iron and magnesium was investigated; the results are shown in Table III.

TABLE III

EFFECT OF SULPHURIC ACID ON THE ATOMIC ABSORPTION OF ZINC, IRON, COPPER AND MAGNESIUM

Element	Amount of element present, p.p.m.	Absorption in absence of sulphuric acid, %	Absorption in presence of 2·5 per cent. v/v of sulphuric acid, %	Absorption in presence of 10 per cent. v/v of sulphuric acid, %
Zinc	0	0·0	0·0	0·0
	1	4·0	2·0	2·0
	4	9·0	9·0	8·0
	8	18·0	16·0	14·0
Iron	0	0·0	0·0	0·0
	20	0·6	0·6	0·4
	80	3·0	2·6	2·0
	160	6·2	5·6	4·2
Copper	0	0·0	0·0	0·0
	2	1·0	0·8	0·8
	8	4·0	4·0	3·0
	16	8·2	7·4	5·6
Magnesium	0	0·0	0·0	0·0
	10	72·0	72·0	60·0
	40	96·4	96·0	94·0
	80	97·8	98·0	97·6

ANALYSIS OF PLANT MATERIAL—

In view of the fact that the only definite interference would be that of residual sulphuric acid from the digestion of plant material, the following procedure for preparation of the sample was investigated. Between 1 and 2 g of oven-dried plant material were digested in 8-inch × 1-inch Quickfit test-tubes with 4 ml, accurately measured, of sulphuric acid - perchloric acid mixture (1 + 7) and about 15 ml of nitric acid. More nitric acid was added if the destruction of organic matter was incomplete after the mixture had been evaporated to a small volume. When the organic matter had been completely destroyed, the digest was heated strongly to the stage at which all the nitric and perchloric acids had been driven off and the 0·5 ml of sulphuric acid remained. The digest was cooled, and 9·7 ml of water were added to make the final volume to 10 ml. Ground-glass stoppers were placed in the test-tubes, which were then heated in a water bath for 30 minutes with intermittent shaking. After cooling and chilling to below room temperature with an ice - water mixture to prevent possible crystallisation after filtration, the solution was filtered through a Whatman No. 42 filter-paper, and 7-ml portions of the filtrate were transferred to Lundegårdh spray bulbs for direct atomic-absorption analysis.

Standards containing 0, 1, 2, 4, 8, 16 and 32 p.p.m. of zinc in 5 per cent. v/v sulphuric acid were prepared, and 7 ml of each were placed in Lundegårdh spray bulbs for absorption measurement and subsequent preparation of a calibration curve.

The sample solutions were analysed first, and then the standards, after which the analysis of several of the earlier samples was repeated to ensure that no drift in sensitivity had occurred during the series of tests. A calibration curve, plotted from the measurements on the standards, was used to determine the concentrations of the sample solutions. A typical calibration curve for zinc is shown in Fig. 1.

COMPARISON WITH POLAROGRAPHIC ANALYSES—

Table IV shows polarographic and atomic-absorption results for zinc in stem, leaf and petiole samples from clover. The polarographic analyses were carried out by Walkley's method.[5]

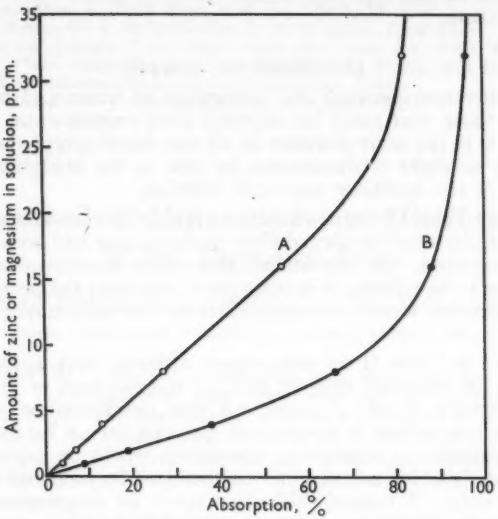


Fig. 1. Atomic-absorption calibration curves for the analysis of plant material for zinc and magnesium. The lines used are Zn 2139 A and Mg 2852 A: curve A, zinc; curve B, magnesium

TABLE IV

ZINC DETERMINATION BY ATOMIC-ABSORPTION AND POLAROGRAPHIC METHODS

Amount of zinc found in clover stem by—		Amount of zinc found in clover leaf by—		Amount of zinc found in clover petiole by—	
atomic absorption, p.p.m.	polarography, p.p.m.	atomic absorption, p.p.m.	polarography, p.p.m.	atomic absorption, p.p.m.	polarography, p.p.m.
27·5	28·2	49·5	47·3	29·5	30·5
27·5	28·8	48·5	47·7	49·0	54·4
25·5	28·8	51·5	55·1	—	—
21·5	22·8	—	—	—	—

Mean of atomic-absorption results = 36·7 p.p.m.
Mean of polarographic results = 38·2 p.p.m.

TABLE V

RECOVERY OF ZINC FROM MATERIAL OF PLANT ORIGIN BY ATOMIC ABSORPTION

Sample	Approximate weight of dry sample, g	Amount of zinc originally present, μg	Amount of zinc added, μg	Amount of zinc found, μg	Recovery, %
Phalaris tops	0·8	127	118	255	108
Wheat heads	2·0	44	118	164	102
White clover leaf ..	0·8	82	118	198	98
White clover straw ..	1·8	90	118	202	95
Oat straw	1·3	30	118	152	103
Sheep faeces	0·9	123	118	240	99

RECOVERY EXPERIMENTS FOR ZINC—

The results of a series of recovery tests for zinc on a variety of materials of plant origin are shown in Table V. These tests were carried out by coning and quartering samples of dry material, combining opposite quarters and digesting the two portions so obtained after an appropriate amount of zinc had been added to one of them. The digests were analysed by the proposed method.

DISCUSSION OF RESULTS

Although the differences between zinc absorption in water and in synthetic plant-ash solutions are within those that could be expected from experimental error, it can be seen that the discrepancy is in the same direction at all zinc levels except the highest (see p. 656). This is probably due to slight contamination by zinc in the analytical-reagent grade salts used in preparation of the synthetic plant-ash solution.

It can be seen from Table II that the concentrations of iron and copper are approximately in the range that would be found in normal plant material that had been prepared for analysis by the procedure described. As the atomic-absorption measurements on these solutions were generally too low to be reliable, it is considered that both the proposed method and the apparatus, in their present forms, are unsatisfactory for determining copper and iron in plant material.

The results shown in Table II for magnesium, although only applicable to one level of magnesium (6 p.p.m. in solution), suggest that no interferences of plant origin will occur during analysis. As plant digests prepared for zinc determination must be diluted one hundred times before magnesium is determined, no interference by residual sulphuric acid from the digestion reagents on magnesium absorption would be expected (see Table III). Standards for plant analysis for magnesium can therefore be prepared by dissolving a magnesium salt in water only. A typical calibration curve for magnesium is shown in Fig. 1. Atomic-absorption readings for magnesium were found to be much more steady than those for zinc, owing, probably, to the effect of variation in supply voltage being less for magnesium emission than for zinc emission from the respective hollow-cathode tubes.

Table II shows that a change in level of some of the absorption results occurs between the figures for calcium and magnesium interferences and also between those for sulphur and aluminium interferences. A shortage of Lundegårdh spray bulbs made it necessary to analyse the solutions in batches of thirty-six, zinc, copper and iron being determined in one batch before analysis of the next batch. The change in level of results was caused by an alteration in the sensitivity of the apparatus when it was changed from analysis for one element to another and then re-set on the first element.

The results for magnesium in Table II indicate that a discernible, but insignificant, drift occurred over the whole series of determinations. This is not surprising when it is considered that the series took more than 2 hours to complete. If the number of analyses in a batch of samples were kept to twenty or less, such a drift would not affect the results.

As the results of polarographic and atomic-absorption analysis are in agreement, choice between the two methods must be based on rapidity and freedom from possible sources of contamination. Analysis by atomic-absorption spectroscopy is superior in both these respects.

The recovery experiments indicate that the proposed procedure for digestion of plant material and subsequent dissolution of the zinc is satisfactory; they also give an additional check on accuracy.

It can be seen from Fig. 1 that the curves for zinc and magnesium are approximately linear up to 18 and 8 p.p.m., respectively. Experience has so far shown that these upper limits of accurate analysis for zinc and magnesium are adequate for the analysis of plant material that has been prepared in the manner described. The lower limits of reasonably accurate analysis for zinc and magnesium in solution were found to be about 0·5 and 0·2 p.p.m., respectively.

I thank Mr. C. H. Williams for the polarographic analyses quoted, Mr. A. Walsh for supplying the electronic equipment[9] used and both for valuable discussion during the work.

REFERENCES

1. Walsh, A., *Spectrochim. Acta*, 1955, **7**, 108.
2. Russell, B. J., Shelton, J. P., and Walsh, A., *Ibid.*, 1957, **8**, 317.
3. Massey, H. F., *Soil Sci.*, 1957, **83**, 123.
4. Cowling, H., and Miller, E. J., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 145.
5. Walkley, A., *Australian J. Exptl. Biol. Med. Sci.*, 1942, **20**, 139.
6. Verdier, E. T., Steyn, W. J. A., and Eve, D. J., *J. Agric. Food Chem.*, 1957, **5**, 354.
7. Jackson, R. H., and Brown, J. G., *Proc. Amer. Soc. Hort. Sci.*, 1956, **68**, 1.
8. Mitchell, R. L., "The Spectrographic Analysis of Soils, Plants and Related Materials," *Tech. Comm. No.* 44, Commonwealth Bureau of Soil Science, 1948.
9. Box, G. F. H., Russell, B. J., and Walsh, A., to be published.

Received *June 5th*, 1958

4:4′-Substituted 2:2′-Dipyridyls in Chelation Reactions With Ferrous Iron

BY G. FREDERICK SMITH AND WM. M. BANICK

(*Noyes Chemical Laboratories, University of Illinois, Urbana, Illinois, U.S.A.*)

The molecular extinction coefficients and wavelengths of maximum absorption in the visible and near ultra-violet part of the spectrum have been determined for the ferrous complexes of eleven new 4:4′- derivatives of 2:2′-dipyridyl, *viz.*, those with $-CH_3$, $-C_2H_5$, –Br, –Cl, $-OCH_3$, $-OC_2H_5$, $-OC_6H_5$, $-COOC_2H_5$, $-CONH_2$, –COOH and $-NO_2$ as substituent. Division of these complexes into three groups according to the shape of their absorption spectra is suggested.

In general, spectrophotometric absorption by the uncomplexed ligands is negligible at the wavelengths of maximum absorption in the ultra-violet region for the ferrous complexes; this is in contrast with the substituted 1:10-phenanthrolines as a class. Possible applications to analysis are discussed.

THIS paper continues the study of the iron^{II} complexes formed by chelating agents containing the grouping =N–C(=)–C(=)–N= and their use in analysis. Substitution in the 3-, 4-, 5- and 6-positions in both rings of the 2:2′-dipyridyls changes the properties of the chelate formed with iron^{II}. These changes are not the same as those brought about by similar substitutions in the 3-, 4-, 5-, 6- and 7- positions in the 1:10-phenanthrolines. The results of substitutions in the 6:6′- positions in dipyridyls and the 2:9- positions in 1:10-phenanthrolines are comparable; chelation with iron^{II} to form a colour is inhibited in both cases. The most consistent modifications in properties for the whole series of dipyridyls, diquinolyls, tripyridyls and 1:10-phenanthrolines are brought about by substitutions in the 4- and 4:7- positions (*para* to ring nitrogens). The ferroine and cuproine reactions (chelation with iron^{II} and copper^{I}) differ markedly and in a predictable manner.

NEW 4:4′- DISUBSTITUTED 2:2′-DIPYRIDYLS STUDIED

The formation of the iron^{II} complex cations and the way their physical constants vary have been investigated for dipyridyls substituted in the 4:4′- positions with—

$-CH_3$,	$-C_2H_5$	–Br,	–Cl,	$-OCH_3$,	$-OC_2H_5$,	$-OC_6H_5$,	$-COOC_2H_5$,	$-CONH_2$,	–COOH and	$-NO_2$
I,	**II**,	**III**,	**IV**,	**V**,	**VI**,	**VII**,	**VIII**,	**IX**,	**X** and	**XI**

PREVIOUS STUDIES OF SUBSTITUTED DIPYRIDYLS

The ferroine reaction of 3-methyl-2:2′-dipyridyl yields a complex of lower stability than the unsubstituted 2:2′-dipyridyl, and 3:3′-dimethyl-2:2′-dipyridyl forms a complex stable only in a narrow range of pH.[1] 3:3′-Dicarboxyl substitutions completely inhibit the formation of a coloured chelate with iron^{II}.[2,3] Single substituent groups in the 6- position diminish, and in the 6:6′- positions completely inhibit, the ferroine reaction, but permit the cuproine reaction.[1,4,5] Dipyridyls substituted in the 5:5′- positions with $-NO_2$, –Br or –Cl groups do not give a ferroine reaction.[6]

Substituent groups such as $-C_6H_5$ in the 4:4′- positions of 2:2′-dipyridyls (*para* substitutions to nitrogen atoms) produce marked changes in property,[7] and this is also true for the three other types of organic ligand, 1:10-phenanthrolines, 2:2′-diquinolyl and 2:2′:2″-tripyridyl.[1,7,8] Further 4:4′- substituted 2:2′-dipyridyls with a variety of substituent groups have been studied in an endeavour to provide data for the selection of the groupings most likely to produce improved reagents when substituted into other types of ligand containing the cuproine or ferroine functional group, such as the tripyridyls. Such compounds might then be synthesised.

Experimental

Reagents—

Standard iron solution—A solution of ferric chloride containing 0·1108 mg of iron per g of solution.

Hydroxylamine hydrochloride—A 10 per cent. aqueous solution freed from iron and copper by treatment with bathophenanthroline and extraction with *iso*amyl alcohol.

Ethanol, 95 per cent.—Analytical-reagent grade, for preparing solutions.

iso*Amyl alcohol*—Analytical-reagent grade, for extractions.

Buffer solutions—Solutions 1·0 *M* in the first named of the following pairs of substances—

pH 1	Potassium chloride - hydrochloric acid
pH 1·9	Potassium chloride - hydrochloric acid
pH 3·1	Acetic acid - sodium acetate
pH 4·0	Acetic acid - sodium acetate
pH 5·7	Sodium acetate - acetic acid
pH 7·1	Ammonium acetate
pH 8·3	Ammonium chloride - ammonium hydroxide
pH 8·8	Ammonium chloride - ammonium hydroxide
pH 9·4	Ammonium chloride - ammonium hydroxide
pH 9·4 to pH 12	Ammonium chloride - sodium hydroxide

Dipyridyl solutions—

0·01 *M* Solutions in ethanol of unsubstituted 2:2′-dipyridyl and of 2:2′-dipyridyls substituted in the 4:4′- positions by $-CH_3$ (**I**), $-C_2H_5$ (**II**), –Br (**III**), –Cl (**IV**), $-OCH_3$ (**V**), $-OC_2H_5$ (**VI**) and $-OC_6H_5$ (**VII**). This last was least soluble in ethanol and had to be treated with sufficient hydrochloric acid to form the hydrochloride.

0·01 *M* Solutions in 50 per cent. ethanol made 1·5 *M* in hydrochloric acid of the $-COOC_2H_5$ and $-CONH_2$ derivatives (**VIII** and **IX**).

A 0·005 *M* solution of the –COOH derivative (**X**) in water to which sufficient ammonium hydroxide was added to complete dissolution.

A 0·0025 *M* solution of the $-NO_2$ derivative (**XI**) in distinctly acidified water.

Optimum pH for formation of chelate complex and its extraction

Before the spectrophotometric constants of the iron^{II} complexes could be evaluated, it was necessary to find the pH for maximum colour development.

To each of a series of test-tubes containing 5·0 ml of buffer solution, 1·0 ml of hydroxylamine hydrochloride solution and 0·25 ml of iron^{III} solution was added 0·5 ml of a 0·01 *M* solution of the ligand. (The ligand solutions that were 0·005 *M* were added in 1·0-ml portions.) Those ligands prepared in 1·5 *M* hydrochloric acid were treated by addition of 0·10 *M* sodium hydroxide in sufficient amount to neutralise the acid. If the complex or the excess of ligand was precipitated, enough 95 per cent. ethanol was added to re-dissolve it. The colours produced at the various pH values were noted. The solution of the complex in each tube was extracted, if possible, by adding 2·0 ml of *iso*amyl alcohol and then shaking the tube (one or two extractions only were applied). Because only a limited amount of ligand **XI**, the $-NO_2$ derivative, was available, it was not included in these tests. No reducing agent other than hydroxylamine was tested. In the buffer solutions of pH 8·8 to 9·4, for three of the ligands studied, there was interference by ammonium hydroxide; when sodium hydroxide was used instead, there was no interference and the maximum colour intensity was attained. The results are shown in Table I.

In the course of obtaining the results in Table I, it was noted that the narrow range of maximum colour formation with the dibromo and dichloro derivatives was due to their low instability constants. Dilution with ethanol augmented this effect. This same effect

has previously been noted for some chloro derivatives of 1:10-phenanthroline.[9] The $-COOC_2H_5$ derivative, which also gave a complex whose colour was stable only over a narrow range of pH, was affected by atmospheric oxidation when extracted into *iso*amyl alcohol. Although the $-C_2H_5$ derivative gives its maximum colour over a wider range of pH than does the $-CH_3$ derivative, it is noteworthy that for the $-OCH_3$ and $-OC_2H_5$ derivatives this effect is reversed. Only the $-OC_2H_5$ derivative gave a ferrous complex that was insoluble in aqueous solution.

TABLE I

INFLUENCE OF pH ON FORMATION AND COLOUR INTENSITY OF IRON^{II} COMPLEXES OF 4:4′- DISUBSTITUTED 2:2′-DIPYRIDYLS AND THEIR EXTRACTABILITY INTO *iso*AMYL ALCOHOL

Ligand	Disubstituted with	pH range for complex formation	pH range for maximum colour	Extractability*
Unsubstituted	—	1·0 to 12	3·1 to 7·1	0
I	$-CH_3$	1·9 to 12	3·1 to 8·3	0
II	$-C_2H_5$	1·9 to 12	3·1 to 12	+
III	–Br	1·0 to 12	1·9 to 3·1	0
IV	–Cl	1·0 to 12	1·0 to 3·1	0
V	$-OCH_3$	1·9 to 12	3·1 to 8·3	+
VI	$-OC_2H_5$	3·1 to 12	4·0 to 8·3	+
VII	$-OC_6H_5$	1·0 to 12	3·1 to 8·3	+
VIII	$-COOC_2H_5$	1·0 to 12	3·1 to 4·0	+
IX	$-CONH_2$	1·0 to 13	4·0 to 12	0
X	–COOH	1·0 to 13	3·1 to 12	0

* Extractability: + = extracted; 0 = not extracted.

DETERMINATION OF SPECTROPHOTOMETRIC CONSTANTS

Ligands **I**, **II**, **V** and **VI** and the unsubstituted dipyridyl were prepared for spectrophotometric examination as follows. Five millilitres of a buffer solution having a pH within the range that gave maximum colour intensity (pH 3·1 to 5·0; see Table I), 2 ml of hydroxylamine hydrochloride solution and 2·0 ml of 0·01 *M* ligand solution were placed in 25-ml calibrated flasks with transfer pipettes. A weighed amount of standard iron solution was added, and the contents of the flasks were made up to volume with water.

The same procedure was followed for ligand **VII**, except that the dilution to volume was with 95 per cent. ethanol.

For ligand **VIII**, the procedure was the same as for ligand **VII**, except that 3·0 ml of the 0·005 *M* ligand solution were taken and a few drops of ammonium hydroxide were added to neutralise the excess of hydrochloric acid.

For ligand **X**, the procedure used for ligand **VII** was followed, except that 3·0 ml of 0·005 *M* solution of ligand were taken.

For ligand **IX**, the procedure used was that for ligand **VIII**, except that the dilution was with water and the excess of reagent that was precipitated was filtered off. The slight tendency for the colour of this complex to diminish on dilution with ethanol made this mandatory.

For ligands **III** and **IV**, 5·0 ml of buffer solution (pH 3·1), 2·0 ml of hydroxylamine hydrochloride solution and 5·0 ml of 0·01 *M* ligand solution were transferred by pipette to 10-ml calibrated flasks. Weighed amounts of iron solution were then added, the complexes were allowed to form, and the contents of the flasks were diluted to volume with 95 per cent. ethanol. The excess of ligand that was precipitated was filtered off before spectrophotometric observations were made.

The iron^{II} complex of ligand **XI** was prepared by taking 5 ml of the 0·0025 *M* solution of the derivative and adding 2·0 ml of hydroxylamine hydrochloride solution and weighed amounts of iron, in a 50-ml calibrated flask. Dilute ammonium hydroxide solution was added until the colour developed, and the flask contents were then diluted to volume with water.

As soon as they had been prepared, the solutions were examined spectrophotometrically with a Cary recording instrument, model 14M. Matched 10-mm silica cells were used for all measurements. A wavelength range of 340 to 650 mμ was covered, and appropriately prepared blank solutions were used for all measurements.

The spectrophotometric results are shown in Table II. Calculations of the molecular extinction coefficient were based upon the amount of iron added, and each value is the average of a series with increasing amounts of iron. (That for ligand **XI** is based upon a single observation.) All complexes gave colours that conformed to Beer's law over the range 1 to 6 p.p.m. of iron.

TABLE II

SPECTROPHOTOMETRIC DATA FOR THE 4:4′- DISUBSTITUTED 2:2′-DIPYRIDYLS AS FERROUS COMPLEXES

Ligand	Disubstituted with	No. of determinations	Wavelength of maximum absorption, mμ	Average molecular extinction coefficient	Wavelength of maximum absorption, mμ	Average molecular extinction coefficient
Unsubstituted	—	4	349	6430	522	8710
I	$-CH_3$	4	354	7860	528	9340
II	$-C_2H_5$	4	355	8410	529	9880
III	–Br	2	357	4960	534	5550
VI	–Cl	2	355	7500	532	8300
V	$-OCH_3$	3	357	7750	525	6680
VI	$-OC_2H_5$	4	359	9100	525	7660
VIII	$-OC_6H_5$	5	363	10,150	540	8060
VIII	$-COOC_2H_5$	5	384	10,380	541	14,150
IX	$-CONH_2$	4	384	11,120	540	14,940
X	–COOH	5	378	10,990	540	14,760
XI	$-NO_2$	1	—	—	525	9190

No diminution in colour intensity on storage for 30 days in glass-stoppered containers was noted for these solutions, except for ligands **III** and **XI**. The colours of these solutions, which had lost much of their original intensity, were not restored by the addition of another portion of reducing agent.

From Table II it will be seen that the absorption peak in the near ultra-violet region (349 to 384 mμ) is common to all these complex cations. This peak was also found by Schilt and Smith[7] for 4:4′-diamino- and 4:4′-diphenyl-2:2′-dipyridyls. There is no comparable absorption peak for 1:10-phenanthrolines, and this serves as a differentiating characteristic.

All the 4:4′- disubstitutions led to increases in the wavelength of maximum absorption as compared with the unsubstituted ligand at both absorption maxima (Tables II and III).

TABLE III

CHANGE IN WAVELENGTH OF MAXIMUM ABSORPTION ($\Delta\lambda_{max.}$) AND MOLECULAR EXTINCTION COEFFICIENT ($\Delta\epsilon$) FOR THE 4:4′- DISUBSTITUTED 2:2′-DIPYRIDYL - IRON^{II} COMPLEXES

Relative to $\lambda_{max.} = 6430$ at 349 mμ and $\epsilon = 8710$ at 522 mμ for the unsubstituted 2:2′-dipyridyl - iron^{II} complex

Ligand	Disubstituted with	Ultra-violet absorption maximum		Visible region absorption maximum	
		$\Delta\lambda_{max.}$, mμ	$\Delta\epsilon$, %	$\Delta\lambda_{max.}$, mμ	$\Delta\epsilon$, %
I	$-CH_3$	+5	+22	+6	+7
II	$-C_2H_5$	+5	+31	+7	+13
III	–Br	+8	−23	+12	−36
IV	–Cl	+6	+17	+10	+5
V	$-OCH_3$	+8	+21	+3	−23
VI	$-OC_2H_5$	+10	+41	+3	−12
VII	$-OC_6H_5$	+11	+58	+18	−7
VIII	$-COOC_2H_5$	+35	+61	+19	+62
IX	$-CONH_2$	+35	+73	+18	+72
X	–COOH	+29	+71	+18	+70
	4:4′-diamino*	+30	+112	+47	−5
	4:4′-diphenyl*	+37	+216	+30	+144

* Taken from the values reported for these ligands by Schilt and Smith.[7]

Ligands **V**, **VI** and **VII** differ from the remainder in that their iron^{II} complex cations have higher molecular extinction coefficients in the near ultra-violet than they possess in

the visible portion of the spectrum. To this group should be added 4:4′-diamino-2:2′-dipyridyl.[7]

The molecular extinction coefficients for the iron^{II} chelate of unsubstituted 2:2′-dipyridyl (8710 at 522 mμ and 6430 at 349 mμ) found in this work are in good agreement with values reported by Busch and Bailar,[10] who gave values of 8700 at 522 mμ and 6500 at 348 mμ. The re-determination of the molecular extinction coefficient of ligand **I** gave a value 10 per cent. higher than that reported at 528 mμ by Cagle and Smith.[2]

In Table III are shown the changes in wavelength of maximum absorption and in molecular extinction coefficient brought about by the substitutions in the various ligands. It can be seen that—

(*a*) the spectra for all the complex cations exhibit bathochromic shifts in the maximum absorption in both the ultra-violet and visible regions;
(*b*) diphenyl substitutions produce the greatest alteration of $\lambda_{max.}$ and ϵ values (compare Table II), an effect that also occurs for similarly positioned phenyl-group substitutions in diquinolyls, tripyridyls and phenanthrolines;
(*c*) in general, increase in $\lambda_{max.}$ values is accompanied by an increase in ϵ values, and this trend is most consistent for the near ultra-violet absorption; and
(*d*) the last five groups listed in Table III, when substituted with dipyridyl, have the greatest influence on the spectrophotometric constants, a finding that is in agreement with similar experimental results for the corresponding 1:10-phenanthrolines, diquinolyls and tripyridyls.[7] To these five substituent groups ($-COOC_2H_5$, $-CONH_2$, $-COOH$, $-NH_2$ and $-C_6H_5$) the hydroxyl group should be added, as exemplified by Synder's reagent (4:7-dihydroxy-1:10-phenanthroline), as shown by Schilt, Smith and Heimbuch.[11]

Grouping of Reagents by Spectrophotometric Data

Spectrophotometrically, unsubstituted 2:2′-dipyridyl and ligands **I**, **II**, **III** and **IV** are similar. Absorption peaks, both in the ultra-violet and visible (349 to 357 mμ and 522 to 534 mμ) regions, are pronounced, and are sharper than those of the similarly substituted 1:10-phenanthrolines, although less sharp than those of the similarly substituted tripyridyls.[8]

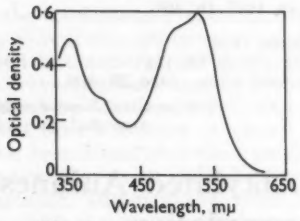


Fig. 1. Absorption spectrum of the 4:4′-dimethyl-2:2′-dipyridyl-iron^{II} complex

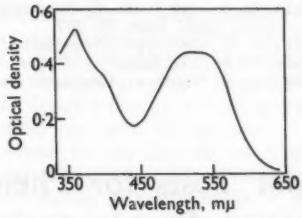


Fig. 2. Absorption spectrum of the 4:4′-diethoxy-2:2′-dipyridyl-iron^{II} complex

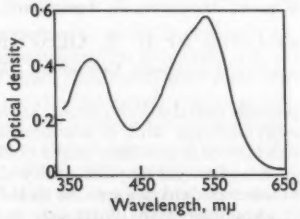


Fig. 3. Absorption spectrum of the 4:4′-dicarboxy-2:2′-dipyridyl-iron^{II} complex

All four ligands have a peak absorption in the ultra-violet region that is 10 to 15 per cent. lower than that in the visible region. Fig. 1 shows an example of this type of absorption spectrum.

Ligands **V**, **VI** and **VII** are again similar in absorption characteristics. The ultra-violet absorption peak is sharp, while that in the visible region is broad. For this group the ultra-violet absorption is the more intense by 17 to 26 per cent. Fig. 2 shows an example of this type.

Ligands **VIII**, **IX** and **X** (see Fig. 3) have similar absorption characteristics to those shown in Fig. 1. They are distinguished by the absence of a shoulder on the short-wavelength peak and by having only a slight indication of a shoulder on the long-wavelength peak.

The possible determination of iron by ultra-violet spectrophotometry

Attempts to use the 1:10-phenanthrolines - iron^{II} complexes for the determination of iron by ultra-violet absorption, with possibly increased sensitivity, have failed owing to interference by absorption at practically the same wavelength from the necessary excess of ligand present.

Similar difficulties occur with the substituted dipyridyls, although in certain circumstances it might be possible to use ligands **II**, **VI**, **VII** and **VIII** (and 4:4′-diphenyl-2:2′-dipyridyl), which absorb negligibly at 340, 330, 340 and 350 (and 356) mμ, respectively, whereas their respective iron^{II} complexes absorb at 355, 359, 363 and 384 (and 386) mμ.

All the ferrous complexes as well as the excesses of ligand are extractable. This makes the proposed scheme of analysis less attractive, except when special conditions owing to other colour interferences are met with.

The syntheses of the newly prepared 2:2′-dipyridyls herein described were carried out by Professor F. H. Case and G. Maerker of Temple University in Philadelphia and will be described elsewhere. Only through this valued assistance in a series of difficult and carefully developed synthetic reaction techniques has this study been made possible.

References

1. Smith, G. Frederick, *Anal. Chem.*, 1954, **26**, 1534.
2. Cagle, F. W., and Smith, G. Frederick, *J. Amer. Chem. Soc.*, 1947, **69**, 1860.
3. Richter, F. P., Doctorate Dissertation, University of Illinois, 1941.
4. Burstall, F. H., *J. Chem. Soc.*, 1938, 1664.
5. Gaines, A., jun., Hammett, L. P., and Walden, G. H., jun., *J. Amer. Chem. Soc.*, 1936, **58**, 1668.
6. Case, F. H., *Ibid.*, 1946, **68**, 2574.
7. Schilt, A. A., and Smith, G. Frederick, *Anal. Chim. Acta*, 1957, **16**, 401.
8. ——, ——, *Ibid.*, 1956, **15**, 567.
9. Schilt, A. A., Doctorate Dissertation, University of Illinois, 1956.
10. Busch, D. H., and Bailar, J. C., jun., *J. Amer. Chem. Soc.*, 1956, **78**, 1137.
11. Schilt, A. A., Smith, G. Frederick, and Heimbuch, A., *Anal. Chem.*, 1956, **28**, 809.

First received *November 26th*, 1957
Amended, *July 16th*, 1958

Spot Tests for Phenols and Alkylated Anilines Based on the Duff Formylation

By F. Feigl and E. Jungreis

(*Laboratório da Produção Mineral, Ministério da Agricultura, Rio de Janeiro, Brazil*)

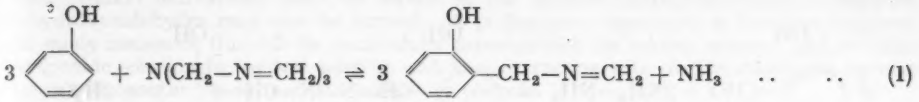
Translated by R. E. Oesper

(*University of Cincinnati, Cincinnati, Ohio*)

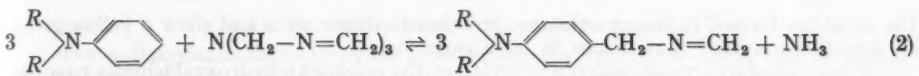
The formylation of phenols and dialkylanilines, as developed by Duff, has been extended to monoalkylanilines, and is accomplished by brief heating at 150° to 160° C with a mixture of crystalline oxalic acid and hexamethylenetetramine. The resulting *o*-hydroxyaldehydes and *p*-dialkylaminobenzaldehydes or *p*-monoalkylaminobenzaldehydes can be detected readily by either production of fluorescent aldazines with hydrazine or formation of orange Schiff's bases with benzidine. These tests for phenols or alkylated anilines come within the scope of spot-test analysis and have microanalytical sensitivity.

Duff[1] has reported that satisfactory yields of *o*-hydroxyaldehydes and *p*-dialkylaminobenzaldehydes can be obtained by formylation of phenols and dialkylanilines with

hexamethylenetetramine (hexamine). The procedures involve two partial reactions. Initially, hexamine reacts in its trialkyl form,[2] and, when heated, participates in the following condensations—

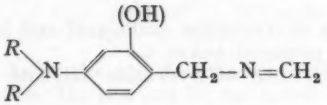


(1)

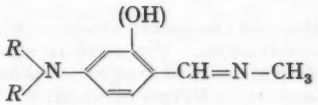


(2)

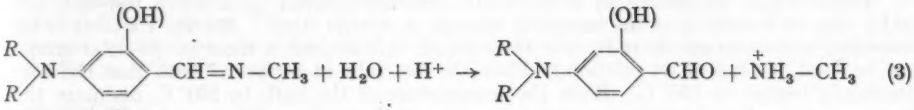
In fact, if hexamine is dry-heated with phenols or dialkylanilines, the evolution of ammonia from the reacting mass can be readily demonstrated by means of appropriate indicator-papers. In the second stage, the condensation products (I) are hydrolysed in their isomeric form (II) by dilute mineral acids to give the respective aldehydes according to reaction (3).



(I)



(II)



(3)

For the condensation reactions, Duff recommends conditions in which hydrolysis of the hexamine is kept at a minimum or even avoided. This is achieved in reaction (1) by heating phenol and hexamine to 150° C for 15 minutes with a mixture of glycerol and boric acid that has previously been kept at 170° C for 30 minutes to remove water. Reaction (2) was accomplished by heating dialkylanilines and hexamine under reflux in a boiling-water bath for 5 hours with a mixture of glacial acetic acid and 90 per cent. formic acid.

It has been found that the formylations, *i.e.*, reactions (1), (2) and (3), occur easily and readily when phenols or dialkylanilines are melted with a mixture of hexamine and oxalic acid dihydrate, although the latter, by virtue of its water of crystallisation, is known[3] to bring about numerous hydrolyses, including the cleavage of hexamine by the following reaction—

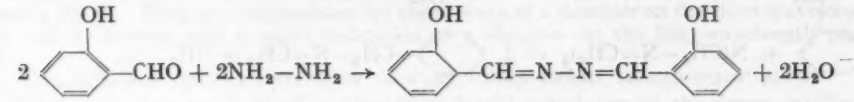
$$(CH_2)_6N_4 + 6H_2O \rightarrow 6CH_2O + 4NH_3$$

It appears, therefore, that reactions (1) and (2), as well as this hydrolysis, occur rapidly. It should likewise be noted that equilibrium reactions are involved (which lead to extensive transformations of phenols and dialkylanilines) and that the reaction products are removed from the equilibrium mixture by the hydrated oxalic acid; ammonia by formation of ammonium oxalate, and condensation products by hydrolysis, as shown in reaction (3). This assumption is supported by the fact that microgram amounts yield the respective aldehydes after heating for 5 minutes at 150° C with oxalic acid and hexamine. Possibly the oxalic acid melt leads to improved yields of aldehydes, but this was not investigated, as our objective was to ascertain whether or not the Duff formylation can be used to detect phenols and alkylated anilines. Satisfactory results were achieved by fusing with oxalic acid, dissolving the reaction mixture in water and testing for aldehyde. The necessary operations are easily conducted by spot-test techniques.

The tests described are additional examples to demonstrate one important point, namely, that suitably modified syntheses and methods of formation can be used as a basis for the detection of organic compounds and functional groups that take part in these changes.[4]

DETECTION OF PHENOLS WITH FREE *ortho*-POSITIONS

To detect *o*-hydroxyaldehydes formed in the Duff formylation, use is made of the immediate condensation of these compounds with hydrazine[5] according to the following reaction—



The aldazines formed in this reaction are resistant to dilute acids and show a yellow-green (or sometimes orange) fluorescence in ultra-violet light.

When phenol and cresols undergo formylation, the resulting *o*-hydroxyaldehydes vaporise at the reaction temperature. Such aldehydes can be detected by holding a piece of filter-paper moistened with hydrazine sulphate solution over the melt. A stain, which exhibits yellow-green fluorescence, can be easily discerned even when no more than a trace of volatile aldehyde is produced.

REAGENTS—

Oxalic acid - hexamine mixture—Mix equal weights of crystalline oxalic acid and hexamethylenetetramine. The mixture should be freshly prepared before use.

Hydrazine sulphate reagent solution—Dissolve 10 g each of hydrazine sulphate and sodium acetate in 100 ml of water.

PROCEDURE—

Place several centigrams of oxalic acid - hexamine mixture in a micro test-tube, and add 1 drop of a solution of the sample in ethanol or diethyl ether. Mix the contents of the test-tube, and warm gently to remove the solvent. (If desired, a trace of the solid sample can be used, instead of its solution.) Place the test-tube in a glycerol bath that has been previously heated to 150° C. Raise the temperature of the bath to 160° C, maintain this temperature for 1 to 2 minutes, and then remove the test-tube. When cool, add 1 drop of hydrazine sulphate reagent solution to the reaction mass. Add 1 drop of water, if necessary, shake the suspension, place it on a filter-paper, and dry for a short time. Examine the filter-paper in ultra-violet light. If the sample contained phenols, the stains on the filter-paper exhibit a blue-green, or sometimes orange, fluorescence.

RESULTS—

Use of the proposed procedure resulted in the detection of 0·25 μg each of phenol, α-naphthol and resorcinol, 1 μg of *p*-hydroxydiphenyl, 2 μg of salicylic acid, 5 μg of *o*-hydroxydiphenyl and 15 μg of 2:7-dihydroxynaphthalene. No aldazine-fluorescence reaction was given by di-β-naphthol, naphthoresorcinol, 2:4-dinitroresorcinol or 1:8-dihydroxynaphthalene-1:3:6-trisulphonic acid.

The fact that certain phenols, contrary to expectation, cannot be detected by the proposed procedure shows that no formylation occurred in the *ortho*-position, but this still leaves open the question of the possible introduction of a –CHO group *meta* or *para* to the phenolic –OH group. To test this point, di-β-naphthol, naphthoresorcinol, 2:4-dinitroresorcinol and 1:8-dihydroxynaphthalene-1:3:6-trisulphonic acid were heated to 150° C with hexamine. Ammonia was evolved, which indicates a condensation analogous to reaction (1). When phenols that show no aldazine-fluorescence reaction are melted with oxalic acid - hexamine mixture and the reaction mass is dissolved in water, the addition of benzidine produces a brown-yellow precipitate, which indicates the formation of a Schiff's base of the resulting aldehyde. These experiments show that a formylation has occurred, but, as yet, no information is at hand regarding its position and extent. It is remarkable that loss of ammonia can still be detected when 150 to 200 μg of these phenols are heated to 160° C with hexamine.

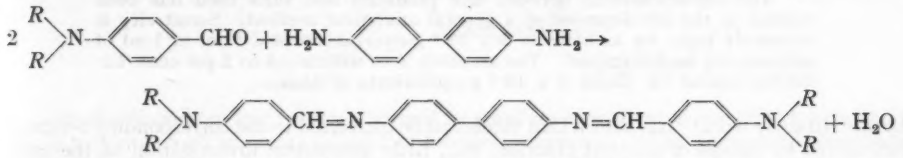
Phenetole and acetylsalicylic acid were tested as representative of phenolic derivatives with free *ortho*-positions. The former gave no aldazine-fluorescence reaction, but the latter responded strongly (0·5 μg was detected). The response of acetylsalicylic acid is obviously due to the fact that, when heated with oxalic acid, hydrolytic splitting-off of acetic acid

occurs with production of salicylic acid. Other phenolic esters may be expected to behave analogously.

The formylation test for phenols is impaired by the presence of aromatic amines and their N-alkyl derivatives, since, as shown by the positive aldazine-fluorescence response, *o*-hydroxyaldehydes may also be formed. A preliminary separation is therefore required. In many instances, this can be successfully accomplished by adding mineral acid or alkali hydroxide solution to the test solution and then extracting with diethyl ether; the aqueous layer will contain the salts of the amines or phenols.

Detection of mono and dialkylanilines

The *p*-dialkylaminobenzaldehydes produced by the Duff formylation can be detected by formation of orange Schiff's bases with benzidine[6] according to the following reaction—



As in the test for phenols, there is no need to isolate the aldehyde after the fusion reaction; the test can be conducted on the aqueous solution of the oxalic acid melt. It is noteworthy that monoalkylanilines (and even aniline itself) can be formylated by heating with oxalic acid - hexamine mixture. It appears that, during formylations, not only are –CHO groups introduced in *para*-positions, but reactions also occur by which amines are converted to phenols. This is shown by the fact that addition of hydrazine salts to an aqueous solution of the oxalic acid melt produces the aldazine-fluorescence reaction of *o*-hydroxylaldehydes.

Procedure—

The procedure is similar to that described for phenols, except that the cooled reaction mixture is treated with 1 to 2 drops of water, and the solution (or suspension) is placed on filter-paper impregnated with a solution of benzidine in diethyl ether. If the test is positive, an orange stain appears, the intensity of which depends on the amount of aromatic aldehyde formed.

Results—

Use of the proposed procedure resulted in the detection of 2 μg each of dimethylaniline, diethylaniline and monomethylaniline and 3 μg of monoethylaniline.

The test cannot be applied directly in the presence of either aldehydes that form coloured Schiff's bases with benzidine or phenols that are formylated by hexamine. If such substances are present, the bases must be separated beforehand; this can be readily accomplished by dissolving the sample in acid and treating the solution with molybdophosphoric acid. The bases can then be recovered from the precipitate by adding alkali and extracting with diethyl ether.

If mono and dialkylanilines are to be detected in the presence of aniline, the aniline should be converted to phenol by warming with nitrous acid (alkali nitrite *plus* hydrochloric acid). If the solution is then treated with alkali hydroxide solution and shaken with diethyl ether, the ethereal solution can be tested for mono and dialkylanilines by the proposed procedure.

References

1. Duff, J. C., *J. Chem. Soc.*, 1941, 547; 1945, 276.
2. Lösekann, G., *Chem. Ztg.*, 1890, **14**, 1409.
3. Feigl, F., Jungreis, E., and Stark-Mayer, C., *Anal. Chim. Acta*, in the press.
4. Feigl, F., "Spot Tests in Organic Analysis," Fifth Edition, Elsevier Publishing Co., Amsterdam and New York, 1956, Chapter 1.
5. ——, *op. cit.*, p. 185.
6. Chakravarti, S. N., and Roy, M. B., *Analyst*, 1937, **62**, 603.

Received *March* 21*st*, 1958

A Scheme for the Colorimetric Determination of Microgram Amounts of Thiols

By B. SAVILLE*

(*Chemical Defence Experimental Establishment, Porton Down, Salisbury, Wilts.*)

A method has been found of converting thiols, and other molecules possessing –SH groups, to their S-nitroso derivatives, which yield an equivalent of nitrous acid on mercuric ion-assisted hydrolysis. This nitrous acid is finally used in the formation of a brilliant azo dye from sulphanilamide and N-1-naphthylethylenediamine.

The correspondence between dye produced and thiol used has been utilised in the development of a general analytical method. Sensitivity is extremely high, for as little as 2×10^{-8} g-equivalents of a thiol in 1 ml of solution can be determined. The accuracy is to within ± 1 to 2 per cent. for determinations on about 2×10^{-7} g-equivalents of thiol.

Although early work[1,2] has shown that thiols can be converted to the corresponding S-nitroso derivatives by means of nitrosyl chloride, etc., little systematic investigation of the more simple chemical properties of these compounds has been reported. It has now been found that the S-nitroso derivatives of some of the more familiar thiols, such as ethanethiol, phenylmethanethiol, cysteine, thiophenol and thioglycollic acid, undergo facile hydrolysis to nitrous acid in the presence of mercuric, silver or cupric salts.

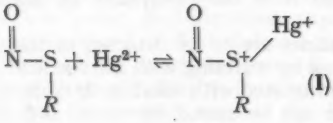
Hence, a solution of S-nitrosocysteine, which can be readily prepared by adding cysteine to excess of sodium nitrite in 0·1 to 1·0 *N* sulphuric acid, is relatively stable ($t_{\frac{1}{2}} \sim 50$ hours) in the presence of ammonium sulphamate, which is added to remove the excess of free nitrous acid; the reactions are as follows—

$$RSH + HONO \rightleftharpoons RSNO + H_2O$$

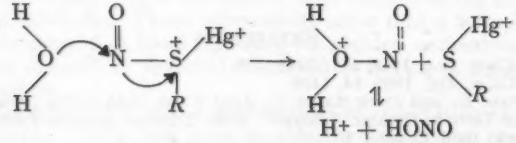
$$\text{Fast} \downarrow \; NH_4SO_3NH_2$$

$$NH_4^+ HSO_4^- + N_2 + H_2O$$

This indicates that the hydrolysis of the S-nitrosothiol (shown by the broken arrow) is slow in acid solution, in contrast to the corresponding behaviour of alkyl nitrites. On the other hand, if a slight excess of mercuric chloride, mercuric acetate or silver nitrate is added to the solution of the S-nitrosothiol prepared in the way described, there is an immediate liberation of nitrogen and the red colour of the nitroso compound is rapidly destroyed. This extremely rapid hydrolysis can be explained by assuming that those metal cations known to possess high affinities for sulphur can engage in rapid reversible co-ordination with the S-nitrosothiol to form a complex (**I**), which, owing to the weakened N–S bond, is then highly susceptible to nucleophilic attack by water molecules. The reactions are as follows—

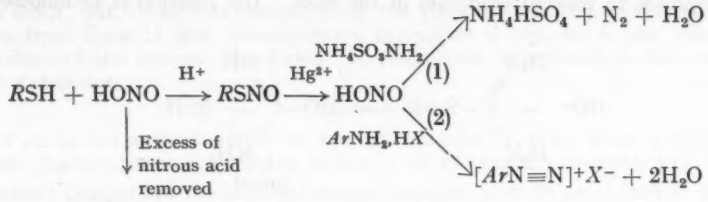


(**I**)



* Present address: The British Rubber Producers' Research Association, 48 Tewin Road, Welwyn Garden City, Herts.

In the experiments described, the liberated nitrous acid immediately reacts with the excess of ammonium sulphamate to form recognisable gaseous nitrogen. However, if sulphanilamide or some other reactive aromatic amine is mixed with the mercuric salt before addition to the S-nitrosothiol solution, the amine competes favourably with the sulphamate for the nitrous acid equivalent to the S-nitrosothiol and gives a high yield of the corresponding diazonium salt. For sulphanilamide, conditions have been found in which diazonium salt formation is almost exclusive, *i.e.*, reaction (2) in the following equation is much more rapid than reaction (1)—



As the diazonium salt (which is formed in amounts equivalent to the thiol taken) can be made to couple with an amine to yield an intensely coloured azo dye, a potential colorimetric method for the determination of thiols was at once realised.

Method

Reagents—

Solution A—Mix 1 volume of a 0·01 *M* aqueous solution of sodium nitrite with 9 volumes of 0·2 to 1·0 *N* sulphuric acid. This solution can be prepared as required.

Solution B—Prepare a 0·5 per cent. solution of ammonium sulphamate in water.

Solution C—Mix 1 volume of a 1·0 per cent. aqueous solution of mercuric chloride with 4 volumes of a 3·4 per cent. solution of sulphanilamide in 0·4 *N* hydrochloric acid.

Solution D—Prepare a 0·1 per cent. solution of N-1-naphthylethylenediamine dihydrochloride in 0·4 *N* hydrochloric acid. This solution must be freshly prepared each day.

Procedure—

To 5 ml of solution A in a 25-ml calibrated flask, add 1 ml of a solution of the thiol (0·00002 to 0·0005 *M*) in water or aqueous ethanol. Set aside for ½ to 5 minutes, according to the nature of the thiol, and then add 1 ml of solution B. Insert the stopper, and shake well for a few seconds to ensure complete removal of excess of nitrous acid. After 1 to 2 minutes, rapidly add 10 ml of solution C (to hydrolyse the S-nitrosothiol and form the diazonium salt), and then make up to the mark with solution D. Colour development is rapid and is usually complete in 3 to 5 minutes with no further change in intensity over a prolonged period. After 10 minutes, measure the coloured solution against an appropriate blank solution with a Spekker absorptiometer and Ilford No. 605 yellow-green filters. (It is advisable to cover the cell containing the blank solution with a cover-glass to prevent access of atmospheric nitrogen oxides.)

Calibration graphs relating absorptiometer reading to thiol concentration can be plotted. These graphs are linear over a wide range of readings and are extremely reproducible.

Discussion of the Method

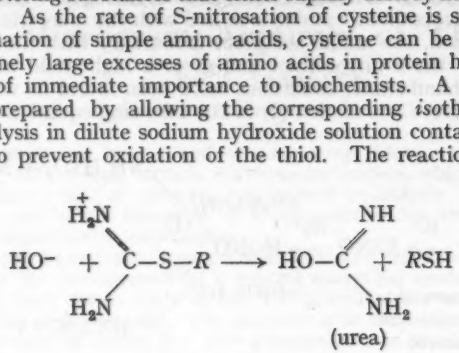
Accuracy—

The accuracy of the method depends on the concentration of thiol being analysed. For 1 ml of 0·00002 *M* thiol, the accuracy is to within ±10 per cent., whereas, for 0·0001 and 0·0004 *M* solutions, the respective accuracies are to within ± 2 to 3 per cent. and ± 1 to 2 per cent.

Interferences, specification and scope—

As far as I am aware, the method is of general applicability to all thiols and has been shown to be specific for thiols in the presence of substances that might be thought, on a rational basis, to interfere. Hence, small amounts of dialkylamines, which could form nitrosamines as a potential source of nitrous acid, do not interfere. This may be because the rate of nitrosation of secondary aliphatic amines is low in acid solution[3] and the rate of hydrolysis

of secondary nitrosamines is high. In general, modifications will need to be introduced to compensate for interfering substances that either rapidly destroy nitrous acid or form coloured nitroso derivatives. As the rate of S-nitrosation of cysteine is several orders greater than the rate of de-amination of simple amino acids, cysteine can be determined without interference from extremely large excesses of amino acids in protein hydrolysates. The method may therefore be of immediate importance to biochemists. A convenient standard thiol solution can be prepared by allowing the corresponding *iso*thiuronium salt to undergo quantitative hydrolysis in dilute sodium hydroxide solution containing about 0·05 per cent. of alkali cyanide to prevent oxidation of the thiol. The reaction is as follows—



I thank R. Bond (vacation student from the University of Glasgow, 1956), H. F. Liddell and Miss P. M. Smyth for assistance and advice during this work.

References

1. Tasker, H. S., and Jones, H. O., *J. Chem. Soc.*, 1909, **95**, 1917.
2. Rheinboldt, H., *Ber.*, 1926, **59**, 1311; 1927, **60**, 184.
3. Taylor, T. W. J., *J. Chem. Soc.*, 1928, 1897.

Received *May 29th*, 1958

The Determination of Traces of Lead and Bismuth in Organic Material

By J. C. GAGE

(*Imperial Chemical Industries Ltd., Industrial Hygiene Research Laboratories, The Frythe, Welwyn, Herts.*)

It has been shown that the method previously described for the determination of lead in organic material may be subject to interference from bismuth, but this can be avoided by reducing to 0·1 *N* the concentration of hydrochloric acid used to extract lead from its diethyldithiocarbamate complex in organic solution. The determination of both lead and bismuth can be made on the same solution by a second extraction of the organic layer with 1·75 *N* hydrochloric acid.

In two previous papers[1,2] a method has been described for determining traces of lead in organic material. It was claimed that the diethyldithiocarbamate separation of lead used in this method holds back any bismuth that may be present and that relatively large amounts of this metal do not interfere in the determination of lead. Experience in other laboratories has not entirely supported this claim, and a further investigation has indicated that the conditions of separating the lead must be more clearly defined in order to remove interference from bismuth. The possibility of adapting the method so that both lead and bismuth can be determined when present together in trace amounts has been investigated.

Experimental

Determination of lead in the presence of bismuth—

In the original method, lead was extracted, after destruction of organic matter by dry ashing, as its diethyldithiocarbamate complex into a mixture of equal volumes of pentanol and toluene. The lead was extracted from the organic layer with dilute hydrochloric acid, which was then added to an alkaline solution of dithizone, and the lead dithizonate complex was extracted with carbon tetrachloride for the colorimetric determination of lead. The dilute hydrochloric acid used to extract lead from its diethyldithiocarbamate complex solution

in pentanol - toluene mixture was prepared by diluting constant-boiling acid (1 + 9) with distilled water; this dilute acid is approximately 0·6 *N*, but different batches show considerable variation. When the concentration of this acid is varied, all other conditions in the method remaining unchanged, there is no effect on the extraction of lead; this is shown by the results in Table I over a range of acid concentrations from 0·05 to 0·65 *N*. The extraction of bismuth from the diethyldithiocarbamate complex solution has, however, been found to be greatly influenced by the concentration of acid used. The results in Table II show that, when 1 mg of bismuth, added as a standard solution of bismuth nitrate in diluted nitric acid (1 + 9), is subjected to the analytical procedure, negligible amounts of bismuth are extracted with acids below 0·5 *N*, but, above this concentration, the amount extracted rises sharply. It can also be seen from Table II that sub-maximum extraction of bismuth is also influenced by the composition of the solvent; the higher the proportion of pentanol in the mixture the greater the extraction.

TABLE I

EFFECT OF ACID CONCENTRATION ON THE EXTRACTION OF LEAD FROM A SOLUTION OF ITS DIETHYLDITHIOCARBAMATE COMPLEX IN PENTANOL - TOLUENE MIXTURE

The optical densities of the lead dithizonate complex from 20 μg of lead in carbon tetrachloride were measured at 515 mμ in a 1-cm cell

Concentration of hydrochloric acid, *N* ..	0·05	0·1	0·25	0·5	0·65
Optical density	0·362	0·341	0·384	0·370	0·361

TABLE II

EFFECT OF ACID CONCENTRATION AND COMPOSITION OF SOLVENT MIXTURE ON THE EXTRACTION OF BISMUTH FROM A SOLUTION OF ITS DIETHYLDITHIOCARBAMATE COMPLEX

The optical densities of the bismuth dithizonate complex from 1 mg of bismuth in carbon tetrachloride were measured at 495 mμ in a 1-cm cell

Composition of solvent mixture		Optical density at different concentrations of hydrochloric acid				
Pentanol, % v/v	Toluene, % v/v	0·1 *N*	0·25 *N*	0·5 *N*	0·65 *N*	0·8 *N*
40	60	0	—	—	0·062	—
50	50	0	0·006	0·025	0·124	0·513
60	40	0·005	—	—	0·20	—

The results in Tables I and II indicate that, when 0·1 *N* hydrochloric acid is used to extract the organic layer, extraction of lead will be complete, but no bismuth will be removed, even when present in large excess.

DETERMINATION OF BISMUTH—

A series of solutions containing 20 μg of bismuth has been submitted to the complete procedure, hydrochloric acid solutions ranging from 0·1 to 2·0 *N* being used to extract the diethyldithiocarbamate complex. The results are shown in Table III. The optical density reached a maximum value with 1·5 *N* acid, and no increase was observed at higher acid concentrations. This maximum optical density has been found to be unaffected by minor variations in the proportions of the solvent mixture. In all subsequent experiments, 1·75 *N* hydrochloric acid was used to extract the bismuth from the first stage of the method. A calibration graph prepared by plotting the optical densities of a series of standard bismuth solutions, measured at 495 mμ in 1-cm cells, against their bismuth contents, gave a straight line with a slope of 0·018 units per μg. The slope of this line is almost identical with that of a calibration graph for lead prepared in a similar manner at 515 mμ.

TABLE III

EFFECT OF ACID CONCENTRATION ON THE EXTRACTION OF BISMUTH

The optical densities of the bismuth dithizonate complex from 20 μg of bismuth in carbon tetrachloride were measured at 495 mμ in a 1-cm cell

Concentration of hydrochloric acid, *N* ..	0·1	0·5	1·0	1·5	1·75	2·0
Optical density	0·031	0·073	0·188	0·329	0·330	0·333

DETERMINATION OF LEAD AND BISMUTH TOGETHER—

To determine traces of lead and bismuth in organic material without including the error due to destruction of organic matter, a sample of tinned spinach was ashed in a silica basin at 450° C, a little magnesium nitrate solution being used to remove final traces of carbon. The ash was dissolved in constant-boiling hydrochloric acid, the solution was evaporated to dryness and the residue was dissolved in the constant-boiling acid diluted (1 + 9) with distilled water to make a solution equivalent to 40 per cent. w/v of spinach. Ten millilitres of this solution were subjected to the analytical procedure, two 10-ml portions of 0·1 *N* hydrochloric acid being used to extract the lead, and then two 10-ml portions of 1·75 *N* hydrochloric acid to extract the bismuth. Each of these extracts was submitted to the final colour-development stage, and the optical density of the dithizone complex in carbon tetrachloride was measured in 4-cm cells. The sample solution was found to contain 1·3 μg of lead in 10 ml, after the reagent blank equivalent to 1·5 to 2 μg of lead had been subtracted; no bismuth was found in the sample or in the reagent blank solution. To 10-ml portions of the sample solution were added known amounts of lead and bismuth, and then the analytical procedure was carried out; with a sample containing 400 μg of lead, it was found necessary to extract three times with 0·1 *N* hydrochloric acid to remove final traces before proceeding to the determination of bismuth. To confirm that iron does not interfere in this determination, an experiment was made in which an excess of iron was present; the results are shown in Table IV.

TABLE IV

RECOVERY OF ADDED LEAD AND BISMUTH FROM SPINACH

Determinations were carried out on 10-ml portions of the spinach extract (equivalent to 4 g of spinach), and each result is the mean of two determinations

Lead added, μg	Bismuth added, μg	Iron added, μg	Lead found, μg	Bismuth found, μg
4	0	0	3·6	—
4	400	0	4·4	—
400	4	0	—	3·25
4	4	0	3·6	4·0
0	0	400	1·2	0
4	4	400	4·4	4·0

DISCUSSION OF RESULTS

The investigation has shown that the concentration of hydrochloric acid used to extract lead from its solution as diethyldithiocarbamate in pentanol - toluene mixture is not critical, but that it must be controlled for the determination of lead in the presence of bismuth and for the subsequent separation of bismuth for colorimetric determination as its dithizone complex.

The experiments with ashed spinach extract, fortified with known amounts of lead and bismuth, have shown that both metals at concentrations equivalent to 1 p.p.m. in the fresh spinach can be determined with adequate accuracy in the presence of 100 p.p.m. of the other metal. Lead and bismuth together, both at a concentration of 1 p.p.m., can be determined on the same solution by successive extraction with 0·1 *N* and 1·75 *N* hydrochloric acid, recoveries being within 10 per cent. of the expected values. The presence of 100 p.p.m. of iron causes no interference in the determination of bismuth, but a slight reduction has been found in the recovery of lead.

Technical assistance in this investigation was provided by Miss Sylvia Morrissey and Mr. T. V. H. Chalker.

REFERENCES

1. Gage, J. C., *Analyst*, 1955, **80**, 789.
2. ——, *Ibid.*, 1957, **82**, 453.

Received *July 8th*, 1958

The Use of Radioactive Phosphorus in the Study of Phosphate Separations

By P. H. BAILEY and R. W. C. BROADBANK
(*School of Chemistry, College of Technology and Commerce, Leicester*)

Phosphate ions containing radioactive phosphorus-32 have been used to study the efficiencies of three procedures for the removal of phosphate ions in semi-micro qualitative analysis. Small amounts of phosphate can be efficiently removed from solution by both the basic acetate and the metallic tin procedure. The zirconium nitrate procedure is also satisfactory, provided that an excess of reagent is avoided. When too much zirconium nitrate was present, separations were poor, owing to cloudiness, and solutions could not be clarified, even by prolonged centrifugation.

Techniques involving radioactive nuclides are of considerable value in investigating analytical procedure, and can often give information about such things as co-precipitation, entrainment or completeness of precipitation. For example, radioactive phosphorus, ^{32}P, provides a particularly simple method of studying procedures for the removal of phosphate ions in qualitative analysis, since, provided that it is in the same chemical state as its inactive isotope, its radioactivity can be used as a measure of the concentration of the latter. Since the beta radiation of phosphorus-32 is fairly energetic ($E_{max.} = 1{\cdot}7$ MeV), a liquid counter, such as that designed by Veall,[1] can be used to measure the activity of labelled phosphate solutions and the comparatively long half-life (14 days) makes corrections for decay during an experiment unnecessary.

Preparation of radioactive solutions—

Carrier-free phosphorus-32, as orthophosphate, in dilute hydrochloric acid can be obtained from the Radiochemical Centre, Amersham, and its metabolism and adsorption by micro-organisms may be prevented by means of an antiseptic. In this work, approximately 1 ml of a 10 per cent. phenol solution was immediately added when an ampoule was opened.

Labelled phosphate solutions were prepared by adding a small amount of carrier-free radiophosphorus (approximately 0·05 to 0·1 microcurie) to standard solutions of calcium phosphate or potassium dihydrogen orthophosphate.

Experimental

Basic acetate procedure—

Two milligrams of calcium orthophosphate in 2 ml of dilute nitric acid were labelled with radioactive phosphate, and dilute hydrochloric acid was added to produce a solution approximately *N* with respect to that acid. Dilute ammonia was added until the solution was neutral to methyl red, and then 2 drops of acetic acid and 20 to 30 mg of solid ammonium acetate were added. Neutralised ferric chloride solution was then added dropwise until the solution turned red, and the tube was set aside in hot water for about 10 minutes. After separation of the precipitate by centrifugation, the volume of the supernatant liquid was adjusted to 10 ml, and the solution was counted in a liquid counter (20th Century Electronics, type M6H). The precipitate was dissolved in a little concentrated hydrochloric acid, the volume was adjusted to 10 ml and the solution was counted in the same counter. After correction of readings for background and lost counts, the percentage of phosphate removed from solution was calculated.

Metallic tin procedure—

Two milligrams of calcium orthophosphate in 2 ml of dilute nitric acid were labelled with radioactive phosphate, and dilute hydrochloric acid was added to produce a solution approximately *N* with respect to that acid. Three to four millilitres of concentrated nitric acid and then 0·1 g of metallic tin were added. After the solution had been carefully evaporated to approximately 1 ml, 5 ml of distilled water were added, and the mixture was stirred and then spun in a centrifuge. The volume of the supernatant liquid was adjusted to 10 ml

and the solution was counted. The residual stannic acid was dissolved in warm concentrated sulphuric acid, the volume was adjusted to 10 ml with this acid, and the solution was counted.

Readings were corrected for background, lost counts and the density of concentrated sulphuric acid, and the percentage of phosphate removed from solution was calculated.

ZIRCONIUM NITRATE PROCEDURE—

Owing to the low efficiencies initially found when this method was used, a much more extensive series of investigations was carried out.

Different volumes and concentrations of phosphate solutions (usually potassium dihydrogen orthophosphate) were labelled with radioactive phosphate, and hydrochloric acid was added to produce a solution 0·9 *N* with respect to that acid. Definite amounts of zirconium nitrate reagent solution[2] were added slowly, and the solution was set aside in the cold for a few minutes. The precipitated zirconium phosphate was separated by centrifugation, and the supernatant liquid was adjusted to a suitable volume and then counted. The precipitate was dissolved in concentrated sulphuric acid, adjusted to the same volume with that acid and then counted. Readings were corrected for background, lost counts and the density of concentrated sulphuric acid, and the percentage of phosphate removed from solution was calculated.

The same procedure was used to study the effects of other ions on the efficiency of the separation, various added salts also being present in the solution of potassium dihydrogen orthophosphate.

CORRECTION FOR DENSITY OF SULPHURIC ACID—

Equal amounts of radioactive phosphate were made up to 10 ml with water and concentrated sulphuric acid, respectively. The two solutions were then counted, and the ratio of the counting rates was used as a correction factor.

CALCULATION OF RESULTS

The method used to calculate the efficiency of separation is shown by the evaluation of the following set of experimental results for the zirconium nitrate procedure—

Volume of solution, ml	= 4
Weight of potassium dihydrogen orthophosphate present, mg	= 20
Amount of zirconium nitrate reagent solution used, drops	= 12
Activity of supernatant liquid, counts per minute	= 121 ± 2
Activity of precipitate, counts per minute	= 8715 ± 66
Background activity, counts per minute	= 17 ± 1
Paralysis-time of counting assembly, microseconds	= 300
Correction factor for density of sulphuric acid	= 1·6

(The standard deviation for the counting rates, N counts being recorded in t minutes, has been taken as $1/t \times N^{\frac{1}{2}}$.)

Counting rate for precipitate (corrected for lost counts), counts per minute

$$= \frac{8715 \times 60}{60 - (8715 \times 3 \times 10^{-6})} = 9100 \pm 67$$

Counting rate for precipitate (corrected for background), counts per minute $= 9083 \pm 67$

Counting rate for precipitate (corrected for density of sulphuric acid), counts per minute $= 9083 \pm 67 \times 1{\cdot}6 = 14{,}533 \pm 108$

Counting rate for supernatant liquid (corrected for background), counts per minute $= 104 \pm 2$

Activity removed from solution, per cent.

$$= \frac{14{,}533 \times 100}{14{,}533 + 104} = 99{\cdot}3$$

This, of course, is the efficiency of the separation.

Owing to the low counting rates for the supernatant liquid and the background, the standard deviation of the percentage efficiency is approximately $\pm \frac{108 \times 100 \times \sqrt{2}}{14{,}637}$, *i.e.*, ± 1 per cent. The efficiency of separation is therefore 99·3 ± 1 per cent. All standard deviations were of the order of ± 1 per cent.

Results

Two experiments were carried out on both the basic acetate and the metallic tin procedures, 2 mg of calcium orthophosphate being present in each instance. The efficiencies of separation were 97·9 ± 1 per cent., 98·8 ± 1 per cent., 99·5 ± 1 per cent. and 99·4 ± 1 per cent., respectively. The results of experiments on the zirconium nitrate procedure are shown in Figs. 1, 2, 3 and 4.

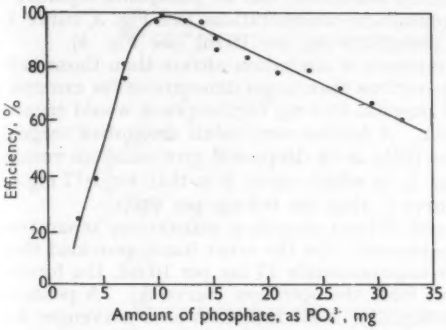


Fig. 1. Efficiency of separating phosphate ions from 10 ml of solution with 12 drops of zirconium nitrate reagent solution

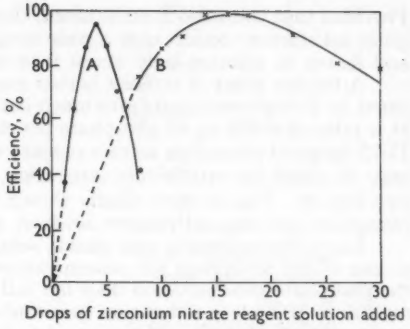


Fig. 2. Efficiency of separating phosphate ions from 10 ml of solution: curve A, 3·5 mg of phosphate, as PO_4^{3-}; curve B, 15·5 mg of phosphate, as PO_4^{3-}

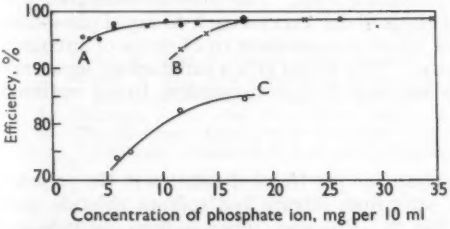


Fig. 3. Efficiency of separating phosphate ions from solution at different ratios of phosphate ion to zirconium nitrate reagent solution: curve A, 0·875 mg of phosphate ion per drop of reagent solution; curve B, 1·17 mg of phosphate ion per drop of reagent solution; curve C, 0·58 mg of phosphate ion per drop of reagent solution

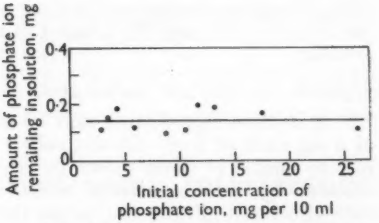


Fig. 4. Amount of phosphate remaining in 10 ml of solution after separation at a ratio of 0·875 mg of phosphate ion per drop of zirconium nitrate reagent solution

Discussion of Results

In absence of added salts—

It can be seen from Fig. 1 that removal of phosphate ions by the zirconium nitrate method is not maintained at optimum efficiency simply by ensuring that excess of zirconium nitrate is present. Cloudy solutions, which could not be clarified even by centrifuging for 30 minutes, were invariably obtained when too much reagent was added. This was presumably caused by colloid formation and was responsible for the low efficiencies under these conditions.

This is further shown by Fig. 2, from which it can be seen that 3·5 mg of phosphate can be removed with optimum efficiency from 10 ml of solution by adding 4 drops only of zirconium nitrate reagent solution. With larger amounts of phosphate there is more latitude, excellent separation of 15·5 mg of phosphate being obtained with 14 to 18 drops of reagent solution.

It is interesting to note that the ratio of phosphate ion per 10 ml of solution to the number of drops of zirconium nitrate reagent solution required for optimum efficiency is approximately constant; this is shown by the following results—

Amount of phosphate ion present, mg per 10 ml	3·5	10·5	15·5
Amount of zirconium nitrate reagent solution needed for maximum efficiency, drops	4	12	14 to 18
Amount of phosphate ion equivalent to 1 drop of reagent solution, mg	0·875	0·875	0·86 to 1·11

Provided that this ratio is maintained, the zirconium nitrate method for phosphate separation gives satisfactory results over a wide range of phosphate concentrations (see Fig. 3, curve A) and leaves in solution only about 0·15 mg of phosphate ion per 10 ml (see Fig. 4).

A further point of interest is that smaller amounts of zirconium nitrate than those indicated by the optimum ratio give much better separations than larger amounts. For example, at a ratio of 0·875 mg of phosphate per drop of reagent, 15·5 mg of phosphate would require 17·75 drops of zirconium nitrate reagent solution. A further very small amount of reagent may be added for satisfactory separation, but as little as 14 drops will give excellent results (see Fig. 2). This is more clearly shown by Fig. 3, in which curve B is that for 1·17 mg of phosphate per drop of reagent solution and curve C that for 0·5 mg per drop.

The latter conditions give cloudy solutions and did not provide a satisfactory separation at any of the phosphate ion concentrations investigated. On the other hand, provided that the phosphate concentration does not fall below approximately 17 mg per 10 ml, the former conditions provide a separation indistinguishable from the optimum (curve A). A possible explanation is that the zirconium phosphate precipitate itself functions as a scavenger for phosphate ions.

In order to express the optimum ratio as phosphate ions per atom of zirconium, 12 drops of zirconium nitrate reagent solution were evaporated on platinum foil, and, on ignition, were found to give 20·0 mg of zirconium oxide. This corresponds to 1·23 mg of zirconium per drop, from which it can be calculated that the optimum reagent to phosphate ratio is almost exactly three zirconium atoms to two phosphate ions. This may be compared with Pittman's recommendation[3] of 35 ml of 0·015 *M* zirconyl chloride for each 40 mg of phosphate, *i.e.*, five zirconium atoms to four phosphate ions, which is equivalent to 15 drops of zirconium nitrate reagent solution per 15·5 mg of phosphate. This would give a satisfactory separation (see Fig. 2), although the amount of reagent is less than that corresponding to our optimum volume.

Effects of certain added ions—

A series of experiments with 3·5 mg of phosphate ion per 10 ml of solution in the presence of 5 mg each of ferric nitrate, cobalt chloride, strontium nitrate and sodium chloride gave results similar to those shown by curve A of Fig. 2. However, when sodium chloride was replaced by sodium tartrate (which will, under suitable conditions, produce a precipitate with zirconium nitrate) the curves shown in Fig. 5 were obtained. These curves are similar to those of Fig. 2, but the optimum amounts of zirconium nitrate solution are 6 drops instead of 4 and 17 to 24 drops instead of 14 to 18. It appears, therefore, that the phosphate and tartrate ions are competing for the zirconium reagent. It may be noted, incidentally, that the amount of reagent recommended by Pittman is no longer sufficient to give a phosphate separation of optimum efficiency.

Zirconium nitrate solution will often give a precipitate in the presence of a large excess of potassium sulphate. A series of experiments was therefore carried out with 3·5 mg of phosphate ion and 20 mg of potassium sulphate, together with other salts, per 10 ml of solution. The results are shown in Fig. 6, from which it can be seen that the optimum amount of reagent is again 4 drops, as in Fig. 2, but the efficiency of separation decreases much less rapidly as excess of zirconium nitrate is added.

Conclusions

From the limited number of determinations carried out, it appears that small amounts of phosphate can be efficiently removed from solution in semi-micro qualitative analysis by either the basic acetate or the metallic tin method. A more comprehensive series of experiments indicates that the zirconium nitrate method can also give excellent separations, provided that an excess of reagent is avoided. From this point of view, the recommendations

in a recently published scheme for semi-micro qualitative analysis[4] are excellent—". . . add zirconium nitrate reagent drop by drop until precipitation is complete. Only a small excess of reagent should be present and it is preferable to add it a few drops at a time, centrifuging after each addition."

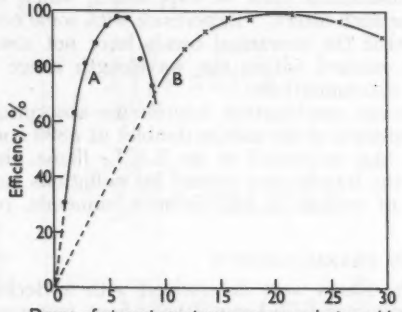


Fig. 5. Effect of the presence of 5 mg each of ferric nitrate, cobalt chloride, strontium nitrate and sodium tartrate on the efficiency of separating phosphate ion from 10 ml of solution: curve A, 3·5 mg of phosphate ion; curve B, 15·5 mg of phosphate ion

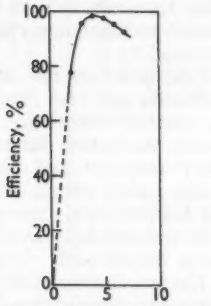


Fig. 6. Effect of the presence of 5 mg each of ferric nitrate, cobalt chloride, strontium nitrate and sodium chloride and 20 mg of potassium sulphate on the efficiency of separating 3·5 mg of phosphate ion from 10 ml of solution

References

1. Veall, N., *Brit. J. Radiol.*, 1948, **21**, 347.
2. Vogel, A. I., "A Textbook of Macro and Semi-micro Qualitative Inorganic Analysis," Fourth Edition, Longmans, Green & Co. Ltd., London, 1954, p. 639.
3. Pittman, F. K., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 514.
4. The Midlands Qualitative Inorganic Analysis Committee, "Semi-micro Qualitative Inorganic Analysis," Stanford & Mann Ltd., Birmingham, 1957, p. 35.

Received *April 23rd*, 1958

The Transmission Characteristics of some Interference Filters for use in Flame Photometry

By R. D. Bond and H. C. T. Stace

(*C.S.I.R.O., Division of Soils, Adelaide, South Australia*)

The transmission characteristics of seven combination interference-absorption filters and one gelatin absorption filter for the isolation of the sodium D line have been determined. The filters have been examined for errors caused in the flame-photometric determination of sodium by radiation emitted by other elements at wavelengths within incompletely suppressed transmission bands.

In recent years, interference filters of the metal - dielectric type have to some extent replaced absorption or colour filters for isolating characteristic radiations in the determination of elements by flame photometry. For the isolation of many radiations, these interference filters can be produced with a narrower transmission band and higher maximum transmittance than the best absorption filters. Such characteristics make interference filters suitable for use in flame photometry. However, for the efficient use of these filters, it is essential that other aspects of their transmission characteristics be appreciated.

Normally, interference filters transmit several orders of frequencies or wavelengths, which are integral multiples of some fundamental frequency; the frequencies at which the transmission maxima occur are determined by the structural characteristics of the filter. They also show some transmittance between these maxima, and this "background," as it is termed, is usually somewhat less than 1 per cent. Under ideal operating conditions, the maximum transmittance at the required wavelength may be as high as 50 to 60 per cent. The general transmission characteristics of a typical interference filter have been discussed by Greenland.[1]

For the effective use of interference filters in flame photometry, the unwanted transmission bands and the "background" transmission must be suppressed, which is usually done by auxiliary filters, *e.g.*, suitable absorption filters. Experience with some commercial interference-absorption filters has shown that the unwanted bands have not always been completely removed and that radiations emitted within the wavelength range of these bands may cause serious errors in some determinations.

The transmission characteristics of seven combination interference-absorption filters, which are commercially available for the isolation of the sodium doublet at 5890 and 5896 A, have been determined. The filters were also examined in an E.E.L. flame photometer (Evans Electroselenium Ltd.) to measure the interference caused by radiations emitted by other elements during the determination of sodium in soil extracts, minerals, rocks and similar materials.

Transmission characteristics

The transmission characteristics of the filters were determined with a Beckman DU spectrophotometer at a slit width of approximately 0·1 mm; the salient features of each filter are shown in Table I.

The values for the various characteristics were found to differ somewhat over the surface of the filter, *e.g.*, for filter B, the maximum transmittance of the sodium D line varied from 20 to 30 per cent. and the wavelength of maximum transmittance from 5820 to 5860 A; the values shown in Table I are for a central portion of the filter. Filters A, B, C, D and E were of Scottish manufacture and were supplied as approximately identical filters for the isolation of the sodium doublet. Filters F and G were of German origin and were described as a precision double-line-filter and a double-line-filter, respectively. Filter H was a standard gelatin absorption filter supplied with the E.E.L. flame photometer for the isolation of the sodium doublet; this filter was included for comparison.

The full transmission curves for filters E, F and H are shown in Fig. 1(a). Of the two filters E and F, E transmits no radiations below 5450 A. It does, however, show another peak at 8640 A and has minimum transmittance (0·4 per cent.) between the peaks. The absorption filter in this combination cuts out only those radiations below 5450 A. Filters A, B, C and D showed transmission characteristics similar to filter E. Filter F, on the other hand, transmits no radiation below 5650 A or above 6500 A. The absorption filter incorporated in F cuts out all radiations below and above these wavelengths. Also, it is noteworthy that filter F has a narrower transmission band than filter E. Filter G was somewhat similar to F in that there was no other transmission peak at longer wavelengths; however, there was a small amount of background transmittance at wavelengths longer than 6500 A. The gelatin absorption filter, H, does not completely cut off the longer wavelengths and has a marked increase in transmittance at 8500 A.

Interferences in the determination of sodium

In the flame-photometric determination of sodium, many other elements and compounds may be present, some of which, *e.g.*, lithium, potassium, rubidium, caesium and calcium oxide, emit radiations in the wavelength range 5500 to 9000 A. The positions of these radiations are shown in Fig. 1 (b). It can be seen that filters E and H transmit different proportions of these radiations. When these elements are present in samples from which the sodium radiation is to be isolated, some interference can be expected. Filter F, on the other hand, does not transmit any of the radiations from lithium, potassium, rubidium and caesium, and interference from these elements should be negligible. It does, however, transmit a small proportion of the radiation from calcium oxide, and some interference from any calcium present in the sample is to be expected. However, because its transmittance at this wavelength is only a small fraction of that of the other two filters (see Fig. 1), this error should be correspondingly smaller.

TABLE I

TRANSMISSION CHARACTERISTICS OF THE FILTERS

	Peak at sodium D							Second peak*		
Filter	Maximum transmittance, %	Wavelength of maximum transmittance, A	Band width at 50 per cent. of maximum transmittance, A	Wavelength of zero transmittance, A	Transmittance at 6100 A, %	Transmittance at 6500 A, %	Transmittance at 7000 A, %	Transmittance at 8000 A, %	Maximum transmittance, %	Wavelength of maximum transmittance, A	Band width at 50 per cent. of maximum transmittance, A
A	17	5900	90	5600	0·8	0·1	0·05	0·15	14	8580	100
B	26	5850	150	5400	3	0·8	0·5	0·6	19	8550	200
C	35	5860	150	5400	3·5	0·6	0·4	1·0	30	8520	180
D	28	5840	160	5400	3	0·4	0·2	0·8	22	8530	200
E	34	5900	200	5450	6	0·8	0·4	0·8	29	8640	200
F	28	5905	90	5650	0·6	<0·01	<0·01	<0·01	—	—	—
G	24	5880	100	5650	0·2	0·05	0·05	0·05	—	—	—
H	9·5	5850	—	5250	3	0·3	0·15	0·45	—	—	—

* No second peak was detected for filters F and G.

TABLE II

APPARENT AMOUNTS OF SODIUM DETERMINED IN VARIOUS SOLUTIONS BY USING DIFFERENT FILTERS

Each solution contained 20 milli-equivalents of solute per litre. The corrected results have been adjusted for the presence of sodium as impurity in the reagents

	Lithium chloride solution		Potassium nitrate solution		Rubidium chloride solution		Caesium chloride solution		Calcium chloride solution	
Filter	Uncorrected amount of sodium found, milli-equivalents per litre	Corrected amount of sodium found, milli-equivalents per litre	Uncorrected amount of sodium found, milli-equivalents per litre	Corrected amount of sodium found, milli-equivalents per litre	Uncorrected amount of sodium found, milli-equivalents per litre	Corrected amount of sodium found, milli-equivalents per litre	Uncorrected amount of sodium found, milli-equivalents per litre	Corrected amount of sodium found, milli-equivalents per litre	Uncorrected amount of sodium found, milli-equivalents per litre	Corrected amount of sodium found, milli-equivalents per litre
A	0·022	0·014	0·008	0·004	0·023	0·007	0·058	0·026	0·050	0·050
B	0·027	0·019	0·026	0·022	0·041	0·025	0·064	0·032	0·132	0·132
C	0·025	0·017	0·023	0·019	0·040	0·024	0·068	0·036	0·121	0·121
D	0·022	0·014	0·020	0·016	0·036	0·020	0·065	0·033	0·109	0·109
E	0·020	0·012	0·011	0·007	0·025	0·009	0·054	0·022	0·114	0·114
F	0·008	Nil	0·004	Nil	0·016	Nil	0·032	Nil	0·033	0·033
G	0·012	0·004	0·007	0·003	0·019	0·003	0·034	0·002	0·039	0·039
H	0·018	0·010	0·007	0·003	0·029	0·013	0·074	0·042	0·133	0·133

In order to determine the magnitude of these expected interferences, solutions containing 20 milli-equivalents per litre of lithium, potassium, rubidium, caesium and calcium were prepared. As lithium and sodium chlorides recrystallise from water together, analytical-reagent grade lithium chloride may contain up to 0·3 per cent. of sodium chloride. Sodium chloride is, however, almost insoluble in *n*-butyl alcohol, lithium chloride being soluble to the extent of 11 per cent. A purified sample of lithium chloride was therefore prepared by forming a saturated solution in *n*-butyl alcohol in the presence of an excess of lithium chloride, spinning in a centrifuge and evaporating the supernatant liquid in a platinum dish. The residue was dissolved in water, the chloride was determined by titration, and the volume was adjusted to give the required concentration.

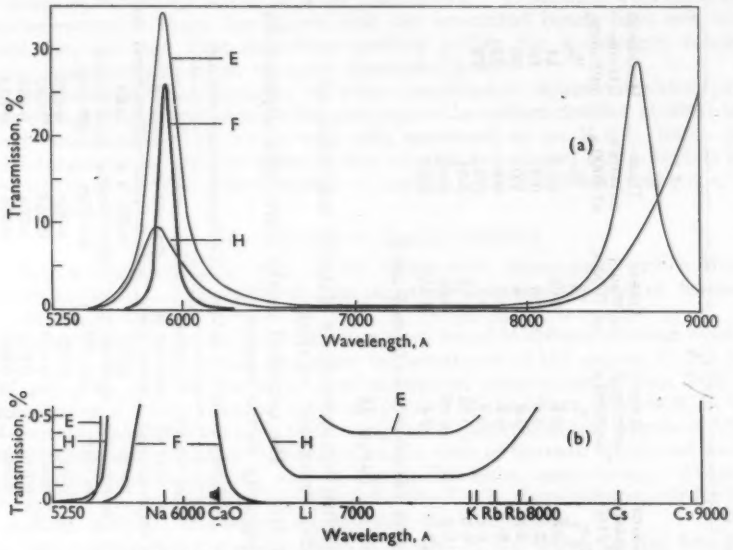


Fig. 1(a). Transmission curves of filters E, F and H

Fig. 1(b). Enlargement of (a) from 0 to 0·5 per cent. transmission

Potassium nitrate, which had been recrystallised and was known to be low in sodium, was used in preference to analytical-reagent grade potassium chloride. From our experience, nitrate in place of chloride at the concentration used has no significant effect on readings with the E.E.L. flame photometer.

The solutions of rubidium and caesium were prepared by dissolving the appropriate amounts of the chlorides in water. The calcium chloride was prepared by dissolving calcium carbonate (of the quality used in the Lawrence-Smith method for determining alkalis) in a slight excess of hydrochloric acid.

Each filter was examined in turn. Sodium chloride solutions of various concentrations in the range 0 to 0·2 milli-equivalent per litre were used to prepare a calibration curve, and the amounts of apparent sodium in the test solutions (all 0·02 *N*) were determined; the results are shown in Table II.

Discussion of results

Each result for apparent sodium in Table II may be the sum of any one or more of three components—

(*a*) An increase in the background radiation at the wavelengths of sodium (5890 and 5896 A) caused by the use of concentrated solutions.
(*b*) Radiation from sodium as impurity in the compounds.
(*c*) Radiation from other elements, which is emitted at wavelengths in the vicinity of the sodium doublet or within other transmission bands that have been incompletely suppressed by the auxiliary filters.

BACKGROUND RADIATION, AND SODIUM AS IMPURITY—

The amount of radiation from lithium, potassium, rubidium and caesium transmitted by filter F (see Fig. 1) is negligible, and results for apparent sodium (see Table II) found with use of this filter for compounds of these elements can be considered to arise from components (*a*) and (*b*).

In order to determine the magnitude of component (*a*), an E.E.L. atomiser-burner was set up in conjunction with a Beckman DU spectrophotometer, and the instrument was calibrated at a slit width of 0·15 mm with a solution containing 1·0 milli-equivalent of sodium per litre. The wavelength scale was then altered (by about 50 A) below sodium 5890 and 5896 A until the transmission scale of the instrument showed for the sodium solution a reading equal to that for distilled water. Each test solution was then atomised, and its background value was determined. For the solutions of lithium, potassium, rubidium and caesium, no increase over the original background value was detected. The results were similar at a wavelength reading above the sodium line. Component (*a*), therefore, did not contribute to the apparent sodium values for compounds of these elements. For calcium, however, the background value varied with the slit width, *i.e.*, some radiation from this element was measurable at sodium 5890 and 5896 A.

From these considerations, it was concluded that the apparent sodium value found for solutions of lithium, potassium, rubidium and caesium when filter F was used were caused by sodium as impurity in the reagents. This was supported by the apparent sodium values for the lithium chloride solution before and after purification, which were 0·047 and 0·008 milli-equivalent per litre, respectively. As the calcium solution was prepared from calcium carbonate that was extremely low in sodium, it is considered that the apparent sodium value found was due to insufficient selectivity of the filters (see Fig. 1), which thus transmitted some radiation from the calcium oxide molecule.

Table II also shows apparent sodium values that have been corrected for the sodium present as impurity.

RADIATION FROM OTHER ELEMENTS—

It is considered that the corrected apparent sodium values in Table II are caused solely by radiation emitted by the particular element under investigation at wavelengths in the vicinity of the sodium line or within other transmission bands that have been incompletely suppressed.

If this is so, the apparent sodium values found for a particular solution with different filters should be approximately proportional to the ratio of the percentage transmission at the wavelength of emission to the percentage transmission at the sodium line. For example, the transmittance of filter E is 34 per cent. at the sodium line and 0·6 per cent. at the lithium line; *i.e.*, the transmittance at the lithium line is 1·8 per cent. of that at the sodium line. For filter H, the transmittance at the lithium line is 1·1 per cent. of that at the sodium line. The error for filter E should therefore be 1·6 times that for filter H. The error in practice is found to be 1·2 times as great, which is satisfactory agreement when it is considered that the transmission characteristics were measured over only a small area of the filter and that variation can occur over the surface of the filter.

When comparing the relative importance of the different elements in any determination, photocell response must be considered. The response of the selenium cell used in the E.E.L. flame photometer decreases at longer wavelengths, and hence the effect of the increase in transmittance of the gelatin filter and the second peaks of some of the interference filters is somewhat offset. However, for instruments in which the newer infra-red sensitive photocells are used, these increases in transmittance could cause greater errors than those reported here.

Although it may be considered that the magnitude of this type of interference is insignificant in many determinations, it could assume serious proportions in some analyses, *e.g.*, the determination of small amounts of sodium in minerals composed largely of the elements considered here. It is therefore essential that the full transmission characteristics of filters used in flame photometers be known, so that precautions can be taken to overcome interferences caused by the transmission of radiation at incompletely suppressed wavelengths.

REFERENCE

1. Greenland, K. M., *Endeavour*, 1952, **11**, 143.

Received *June 9th*, 1958

Notes

THE USE OF SULPHURIC ACID TO DEPRESS THE INTERFERENCE OF CALCIUM IN THE DETERMINATION OF SODIUM WITH AN E.E.L. FLAME PHOTOMETER

THE E.E.L. flame photometer (Evans Electroselenium Ltd.) makes use of coal gas and air to give a relatively low-temperature flame, and absorption filters are used to isolate the appropriate radiations.[1] Under these conditions, some of the radiation from calcium oxide bands may pass through the filter and cause considerable error in the determination of sodium. This error can be reduced by using a more selective filter that transmits less of the calcium oxide radiation and also by decreasing the intensity of the calcium emission. Certain interference filters, which have maximum transmission at 5890 A, transmit less calcium oxide radiation than does the gelatin filter supplied with the instrument, and use of such filters reduces the calcium interference. The complete transmission characteristics of an interference filter must be known, however, before it can be used safely.[2]

A coal gas - air flame supplies insufficient energy to volatilise all calcium salts to the same extent, and this property can be used to reduce the interference further. The well known depressive effect of phosphate on the emission of calcium in such a flame is largely due to the lower volatility and dissociation of calcium phosphate.[3,4,5] When added to solutions of calcium chloride, sulphates also depress calcium emission. Although this depression is less than that caused by phosphates, it is of greater analytical significance in controlling the error caused by calcium in the determination of sodium, for it can be made constant at any given calcium level. The depression caused by phosphate is not constant; it depends on other anions present, and emission is partly restored by sulphate. In the presence of a sufficient amount of sulphate, however, calcium emission is independent of other anions naturally present and is determined solely by the calcium level.

EXPERIMENTAL

To measure the depression in calcium emission caused by adding sulphate, the "calcium" interference filter supplied with an E.E.L. flame photometer was placed in position, and the instrument was adjusted to give a galvanometer reading of 100 with a solution of calcium chloride containing 1·00 milli-equivalent of calcium per litre. The scale readings for solutions of calcium chloride with different amounts of sulphuric acid were then determined; the results are shown in Table I.

TABLE I

EFFECT OF SULPHATE AND PHOSPHATE ON CALCIUM EMISSION

Calcium chloride present, milli-equivalents per litre	Calcium sulphate present, milli-equivalents per litre	Potassium dihydrogen phosphate present, milli-equivalents per litre	Potassium chloride present, milli-equivalents per litre	Orthophosphoric acid present, milli-equivalents per litre	Sulphuric acid present, milli-equivalents per litre	Galvanometer reading
1·00	—	—	—	—	—	100
1·00	—	—	—	—	0·30	83
1·00	—	—	—	—	0·60	71
1·00	—	—	—	—	1·00	65
1·00	—	—	—	—	5·00	64
0·50	—	—	—	—	—	50
0·50	—	—	—	—	0·30	35
0·50	—	—	—	—	0·50	32
1·00	—	—	—	1·00	—	19
1·00	—	—	—	1·00	1·00	63
1·00	—	1·00	—	—	—	20
1·00	—	1·00	—	—	1·00	39
1·00	—	1·00	—	—	2·00	63
—	1·00	—	—	—	—	65
—	1·00	—	—	1·00	—	61
—	1·00	1·00	—	—	—	31
—	1·00	1·00	—	—	1·00	61
—	1·00	—	1·00	1·00	—	23
—	1·00	—	1·00	1·00	1·00	62
—	1·00	—	5·00	1·00	1·00	58
—	1·00	—	5·00	1·00	5·00	65

It can be seen that the calcium emission is progressively decreased until the amount of sulphuric acid added is equivalent to the amount of calcium present, after which further additions of sulphuric acid are without effect. This implies that, when a small excess of sulphuric acid is present in the determination of sodium, not only is the error caused by extraneous calcium emission reduced by approximately one-third at any given level of calcium, but it is reduced to a constant value for that level.

The interaction of sulphate and phosphate on calcium emission in the presence of other salts was also studied, the same interference filter being used. From the results, which are also shown in Table I, it can be seen that, although phosphate reduces calcium emission much more markedly than does sulphate, in the presence of both phosphate and sulphate, the emission is suppressed to a lesser extent depending on the amount of phosphate that remains combined with other metal ions. When phosphate is present and the amount of sulphuric acid used is slightly in excess of that equivalent to the calcium and other metal ions, the calcium radiation corresponds almost exactly to that of a pure calcium sulphate solution of equivalent concentration.

To confirm that the error caused by calcium in the determination of sodium can be controlled by reducing and stabilising calcium radiation in the vicinity of the sodium doublet, the radiation transmitted by "sodium" filters of both gelatin and interference types was measured. The solutions used and the results obtained are shown in Table II.

TABLE II

EFFECT OF CALCIUM AND SULPHURIC ACID ON THE DETERMINATION OF SODIUM

Calcium chloride present, milli-equivalents per litre	Sulphuric acid present, milli-equivalents per litre	Sodium chloride present, milli-equivalents per litre	Galvano-meter reading with gelatin filter (H)	Galvano-meter reading with interference filter (A)	Increase in sodium with filter H, micro-equivalents per litre	Increase in sodium with filter A, micro-equivalents per litre
2·00	—	—	7	3	12	5
5·00	—	—	18	8	34	13
10·00	—	—	36	15	66	26
2·00	1·00	—	5	3	10	4
2·00	2·00	—	4	2	8	3
5·00	1·00	—	16	7	30	12
5·00	5·00	—	10	4	18	7
10·00	10·00	—	17	8	31	13
2·00	—	0·100	63	61	17	8
5·00	—	0·100	74	64	40	16
10·00	—	0·100	91	70	80	30
2·00	2·00	0·100	60	58	12	4
5·00	5·00	0·100	65	61	22	8
5·00	1·00	0·100	72	63	36	14
10·00	10·00	0·100	72	63	37	14

The characteristics of the filters H and A have been determined by Bond and Stace.[2] The apparent amounts of sodium found in the sodium-free solution (caused by extraneous calcium emission) and the amounts in excess of those known to be present in the other solutions are shown in the last two columns of Table II. It can be seen that, when an amount of sulphate equivalent to the calcium present has been added, the error is less than in the absence of sufficient sulphate and also that the error is less with interference filter A than with gelatin filter H.

CONCLUSIONS

In the determination of sodium in dilute solutions containing calcium by means of an E.E.L. flame photometer, a small excess of sulphuric acid should be added to stabilise and reduce calcium emission. The amount of sulphuric acid should be at least equivalent to the amount of calcium present, and, if the solution contains anions, such as phosphate or bicarbonate, an additional amount of sulphuric acid equivalent to any other metal ions present should be added. Under these conditions, with a given filter, calcium radiation can be determined (as shown by the results in Table II) and a suitable correction can be applied to the sodium value if, as often happens, the amount of calcium present is known.

In dilute solutions, this correction is essentially independent of the amount of sodium present, but this is not so for, say, the 0·2 *N* ammonium chloride commonly used in soil analysis. With such solutions it was found that, when interference filter A was used, the correction increased from 0·01 milli-equivalent of sodium per litre for each 10 milli-equivalents of calcium in the absence

of sodium, to 0·02 milli-equivalent of sodium per litre for the same amount of calcium in the presence of 0·10 milli-equivalent of sodium per litre.

References

1. Collins, G. C., and Polkinghorne, H., *Analyst*, 1952, **77**, 430.
2. Bond, R. D., and Stace, H. C. T., *Ibid.*, 1958, **83**, 679.
3. Margoshes, M., and Vallee, B. L., *Anal. Chem.*, 1956, **28**, 180.
4. Scharrer, K., and Jung, J., *Z. PflErnähr. Düng.*, 1954, **67**, 240.
5. Stace, H. C. T., and Hutton, J. T., "Flame Excitation Methods of Spectrochemical Analysis," C.S.I.R.O., Melbourne, 1958.

C.S.I.R.O., Division of Soils
Adelaide, South Australia

R. D. Bond
J. T. Hutton
Received *June 9th*, 1958

A REACTION BETWEEN *meso*INOSITOL AND URANYL ACETATE

Existing chemical methods for the detection and determination of inositol depend on its reducing power as a polyhydroxy compound. A reaction has been observed between *meso*inositol and uranyl acetate solutions, which may provide the basis for a method of detection on paper chromatograms[1] and for determination in solution after chromatographic separation from other polyols.[2]

Qualitative test on paper chromatograms

The chromatogram is sprayed lightly with a 2 per cent. aqueous solution of uranyl acetate and is immediately examined under ultra-violet illumination. The presence of inositol is indicated by a green fluorescence against a colourless background. The normal fluorescence of uranyl acetate is completely suppressed by the paper.

Of possible interfering substances tested, only glycerol was found to react, and then only in milligram amounts. Sugars do not react. There has been no opportunity to test the isomeric inositols. Inositol monophosphate reacts only faintly.

Reaction in solution

By contrast, in aqueous solution (pH 4·4), the large blank fluorescence of uranyl acetate is only feebly enhanced by addition of inositol. When increasing amounts of sodium hydroxide are added, the enhancement of fluorescence caused by inositol is decreased to the point where the effect of inositol is reversed and quenching occurs. This quenching effect is of much greater magnitude than the enhancement and is more suitable for measurement. There would appear to be no theoretical limit to the sensitivity, but, in practice, as the sensitivity increases the accuracy decreases. Quenching of fluorescence by polyhydroxy compounds has been noted by West.[3]

A tentative method, in which the quenching effect is used, has been established for the determination of inositol eluted from paper chromatograms. Standard curves are completely reproducible in the range 20 to 200 μg, but the main difficulty has been the variable blank values found from the paper strips. Inositol was completely extracted from the paper into uranyl acetate solution in 1 hour, and the degree of quenching of the fluorescence was constant 2 hours after the alkali had been added.

Two other aspects of the reaction are worth mentioning. At pH 4·4, addition of inositol to uranyl acetate solution has little effect on the pH, but enhances the fluorescence. At pH 6·0, addition of inositol causes quenching of the fluorescence, and the solution becomes more alkaline. This increase in pH was directly proportional to the amount of added inositol, but was of small magnitude (0·27 pH units per 0·1 mg of inositol).

The yellow complex formed by the reaction of *meso*inositol with uranyl acetate at pH 4·4 showed an apparent absorption curve with peaks at 356, 315 and 240 mμ when a single monochromator instrument was used. However, the sensitivity attainable by absorptiometry at these peaks does not approach that of the fluorimetric quenching method. The formation of coloured complexes between the uranyl ion and polyhydroxy compounds has been noted previously.[4]

References

1. Hawthorne, J. N., *Biochim. Biophys. Acta*, 1955, **18**, 391.
2. Meredith, P., and Sammons, H. G., *Analyst*, 1952, **77**, 416.
3. West, W., "Physical Methods in Organic Chemistry," Second Edition, Interscience Publishers Inc., New York, 1949, Volume I, p. 1441.
4. Rodden, C. J., and Warf, J. C., National Nuclear Energy Series, Division VIII, "Analytical Chemistry of the Manhattan Project," McGraw-Hill Book Co. Inc., New York, 1950, Volume I, p. 12, references 59 and 60 (originals not seen).

Department of Medical Biochemistry
and Pharmacology
University of Birmingham

P. Meredith
H. G. Sammons
Received *July 3rd*, 1958

THE DETERMINATION OF ETHYLENE DIBROMIDE IN BRINE

THE methods generally used for determining halogens in organic compounds, such as the sodium fusion method[1] and the Carius and other combustion methods,[2,3,4,5] can be applied to the determination of ethylene dibromide. However, a more simple procedure especially adapted to simple aliphatic alkyl halides involves alkali hydrolysis and subsequent determination of the liberated bromide.

Kennett[6] described a method for determining ethylene dibromide and ethylene chlorobromide in air. An ethanolic solution of these halides, prepared by shaking the sample of air with ethanol, is heated under reflux in the presence of sodium hydroxide for 15 minutes, and, after acidification with nitric acid, the bromide is determined argentimetrically by Volhard's method.

The proposed method is based on the extraction of ethylene dibromide from the brine obtained during process development for the manufacture of ethylene dibromide from ethylene and bromine, the latter being liberated *in situ* from bromide with chlorine gas. The extraction is carried out with xylene, and the extract is then heated with potassium hydroxide in the presence of *n*-butyl alcohol, which serves as a mutual solvent for the xylene and the alkali. The liberated bromide is determined gravimetrically as the silver salt.

METHOD

REAGENTS—

Xylene—Technical grade.
n-*Butyl alcohol*—The reagent free from halides.
Potassium hydroxide—Analytical-reagent grade.
Silver nitrate solution, 0·1 N.
Nitric acid, diluted (1 + 3)—Prepared from the analytical-reagent grade acid.

PROCEDURE—

Place an aliquot of the brine containing 10 to 200 mg of ethylene dibromide in a 250-ml separating funnel. Add 4 to 5 ml of xylene, and shake the mixture gently for about 3 minutes. Allow the layers to separate, and then run off the lower brine layer into another 250-ml separating funnel and transfer the xylene layer to a 50-ml separating funnel. Repeat the extraction three times with 5-ml portions of xylene. Collect the xylene extracts in the 50-ml separating funnel, and shake six to eight times with 1 to 2-ml portions of water until the washings are chloride-free. Transfer the washed xylene solution to a 100-ml Pyrex-glass test-tube (30 cm long and 2 cm in diameter). Rinse the 50-ml separating funnel twice with 8-ml portions of *n*-butyl alcohol, and add the washings to the xylene solution in the test-tube. Add 4 to 5 g of potassium hydroxide to the test-tube, and close it with a well fitting rubber stopper. Heat the tube in a bath of boiling saturated sodium chloride solution (108° to 110° C) for a few minutes, and then shake the tube gently to saturate the solution with potassium hydroxide (about 2 *N*). Continue heating for 1 hour, and then remove the tube from the bath, and cool it under running water to room temperature. Transfer the contents to a 250-ml beaker with the aid of 100 ml of water. Stir, and, while stirring, neutralise the solution with diluted nitric acid (1 + 3) with methyl orange as indicator, and add 2 ml in excess. Precipitate the bromide with a slight excess of 0·1 *N* silver nitrate solution, collect the precipitate on a fine sintered-glass crucible, wash with water until the washings are free from silver, and, finally, wash 4 or 5 times with a few millilitres each time of *iso*propyl alcohol to remove the xylene. Dry the precipitate at 120° to 130° C, and weigh. Carry out a blank determination on the reagents by heating a mixture of 20 ml of xylene, 16 ml of *n*-butyl alcohol and 4 to 5 g of potassium hydroxide in the same way as for the sample, and deduct the weight of silver halide (if any) from that found in the determination.

If the brine to be analysed contains free halogens, they are extracted and then react immediately with xylene to yield halogen derivatives, which liberate halide ion on treatment with alkali. In such instances, the brine should be treated with an excess of potassium iodide and the liberated iodine titrated with 0·1 *N* sodium thiosulphate solution to convert the free halogen to the halide salt before extraction with xylene.

RESULTS

The proposed procedure has been tested with solutions prepared by dissolving weighed amounts of analytical-reagent grade ethylene dibromide in 200-ml portions of Dead Sea "salt

pan end-brine," which is the mother liquor obtained in the solar concentration of Dead Sea water for the processing of carnallite. The composition of a typical Dead Sea "end-brine" is shown in Table I, and the results of the determinations in Table II.

TABLE I

COMPOSITION OF A TYPICAL DEAD SEA "END-BRINE"

Ion present	Amount present, g per litre
Cl^-	349
Br^-	12·2
Ca^{2+}	47·3
Mg^{2+}	90·4
K^+	0·7
Na^+	17

Sp.gr. at 21° C = 1·340.

TABLE II

DETERMINATION OF ETHYLENE DIBROMIDE IN DEAD SEA "END-BRINE"

Amount of ethylene dibromide in 200 ml of brine, mg	Ethylene dibromide found, mg	Error, %
11·0	10·9	−0·9
29·2	28·9	−1·0
43·2	43·6	+1·0
51·5	51·2	−0·6
78·4	78·9	+0·8
102·2	102·5	+0·3
156·5	156·0	−0·3
198·1	198·0	—

Direct extraction of ethylene dibromide from the brine with *n*-butyl alcohol (or other partially miscible solvents) was not possible owing to the high solubility of magnesium and calcium halides in the alcohol. Extraction with ether and then evaporation of the ether at 50° C in a fractionating column 60 cm long and subsequent alkali hydrolysis in ethanolic medium gave low results (5 to 10 per cent.) owing to losses at the evaporation stage.

When the heating period was reduced to 30 minutes under the conditions described, the results were 4 or 5 per cent. lower.

We thank the Directors of the Laboratories of Israel Mining Industries for permission to publish this Note. The helpful criticism of Dr. Alexander Alon, Chief Analyst, Israel Mining Industries, is also gratefully acknowledged.

REFERENCES

1. Lohr, L. J., Bonstein, T. E., and Frauenfelder, L. J., *Anal. Chem.*, 1953, **25**, 1115.
2. Lawrence, M. W., and Mary, D. K., *Ibid.*, 1950, **22**, 1049.
3. Niederl, J. B., and Niederl, V., "Organic Quantitative Micro-analysis," Second Edition, John Wiley & Sons Inc., New York, 1942.
4. Safford, H. W., and Stragand, G. L., *Anal. Chem.*, 1951, **23**, 520.
5. Haslam, J., *Analyst*, 1950, **75**, 371.
6. Kennett, B. H., *J. Agric. Food Chem.*, 1954, **2**, 691.

ISRAEL MINING INDUSTRIES LABORATORIES
HAIFA, ISRAEL

J. R. MASHALL
D. A. ADER

Received *July* 1*st*, 1958

THE APPLICATION OF JANOVSKY'S AND MOHLER'S REACTIONS TO THE DETECTION OF BENZENE HEXACHLORIDE

Two tests based on Janovsky's[1] and Mohler's[2] reactions for polynitro aromatic compounds are described for the detection of benzene hexachloride.

JANOVSKY'S REACTION

Janovsky's reaction has been widely used for the detection and determination of a variety of aromatic compounds having nitratable benzene nuclei. Janovsky observed that dinitro substitution products of benzene, toluene and naphthalene gave colour reactions with acetone and alkali. The Vitali - Morin[3] test for atropine and related alkaloids, Dolin's[4] colorimetric method for determining benzene and the Schechter - Hornstein[5] method for the detection and determination of benzene hexachloride are instances of the application of Janovsky's reaction.

The method described by Schechter and Hornstein is based on the dechlorination of benzene hexachloride to benzene and nitration of the benzene formed to *m*-dinitrobenzene, which, with ethyl methyl ketone, forms a colour in the presence of alkali. The dechlorination and nitration are carried out in a specially designed all-glass apparatus. Hancock and Laws,[6] who made a critical survey of the method, designed a simpler apparatus in which the nitration is carried out in the cold.

In both methods, however, dechlorination is effected by heating with zinc and glacial acetic acid. In the proposed procedure, the dechlorination and subsequent nitration are carried out in the cold in an extremely simple apparatus, namely, a Cavett flask[7] (see *Analyst*, 1954, **79**, 125). Magnesium, which reacts vigorously with glacial acetic acid in the cold, is used instead of zinc for dechlorinating benzene hexachloride.

PROCEDURE—

A nitration acid is prepared by dissolving 5 g of potassium nitrate in 100 ml of concentrated sulphuric acid. Between 0·5 and 1·0 ml of this acid is placed in the flask, which is then rotated to spread out the acid. A 0·2-ml portion of a solution of benzene hexachloride in glacial acetic acid is placed in the cup, three pieces of magnesium wire, each the size of a pinhead, are then added and the stopper carrying the cup is replaced immediately, 1 drop of the nitration acid being used to lubricate the joint. After 30 minutes, the stopper is removed, the flask is cooled in ice and the nitration acid is transferred to a separating funnel with about 20 to 25 ml of water. The solution is extracted with an equal volume of diethyl ether that has been washed with alkali, and the aqueous layer is discarded. The ether layer is washed with 5 ml of 2 per cent. w/v sodium hydroxide solution and then with 5 ml of a saturated solution of sodium chloride, after which it is filtered through a plug of alternate layers of cotton-wool and anhydrous sodium sulphate, the filtrate being collected in a large test-tube. Four drops of liquid paraffin are added to the filtrate, and the ether is evaporated. The residue is dissolved in 10 ml of a mixture of acetone and absolute ethanol (1 + 1,v/v), 0·1 ml of a 4 per cent. w/v solution of potassium hydroxide in methanol is added, a stopper is placed in the test-tube, and the contents are mixed. A crimson colour develops in about 2 minutes and deepens after the solution has been set aside.

The test is sensitive to 5 μg of benzene hexachloride.

MOHLER'S REACTION

Mohler described a test for benzoic acid depending on the colour formed when the dinitro derivative of benzoic acid is treated with ammonium sulphide in ammoniacal medium. The test was modified by Grossfeld,[8] who substituted hydroxylamine hydrochloride for ammonium sulphide. With this modification, the final colour was purer and more permanent.

PROCEDURE—

The dechlorination and nitration of benzene hexachloride are carried out as described for Janovsky's reaction, but only 0·5 ml of the nitration acid is used. After 30 minutes, the flask is cooled in ice and the nitration acid is diluted with 2·5 ml of water. Five millilitres of 25 per cent. w/w ammonia solution are carefully added, and the solution is mixed. Two millilitres of 2 per cent. w/v hydroxylamine hydrochloride solution are added, the solution is mixed well, transferred to a stoppered test-tube and set aside. A violet colour develops slowly and deepens on standing.

The test is sensitive to 0·25 mg of benzene hexachloride.

I thank the Government Analyst, Mr. G. A. C. Sirimanne, B.Sc., F.R.I.C., for permission to publish this Note.

REFERENCES

1. Janovsky, J. V., *Ber.*, 1891, **24**, 971.
2. Mohler, E., *Bull. Soc. Chim.*, 1890, **3**, 414.
3. Morin, C., *J. Pharm. Chim.*, 1936, **128**, 545; abstracted in *Analyst*, 1936, **61**, 504.
4. Dolin, B. H., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 242.
5. Schechter, M. S., and Hornstein, I., *Anal. Chem.*, 1952, **54**, 544.
6. Hancock, W., and Laws, E. Q., *Analyst*, 1955, **80**, 665.
7. Cavett, J. W., *J. Lab. Clin. Med.*, 1937/38, **23**, 543.
8. Grossfeld, J., *Z. Untersuch. Nahr. Genussm.*, 1915, **30**, 271; abstracted in *Analyst*, 1916, **41**, 97.

GOVERNMENT ANALYST'S LABORATORY
COLOMBO 7, CEYLON

E. RATHENASINKAM

Received *February 10th*, 1958

THE SPECTROPHOTOMETRIC DETERMINATION OF PERRHENATE

A METHOD, which it is hoped will be described in a later paper, is being developed for the determination of rhenium in organic rhenium complexes containing nitrogen. The materials are completely oxidised by fusion with sodium peroxide in a micro Parr bomb, the melt is dissolved in water, and the solution is acidified and boiled to expel carbon dioxide, which gives a solution containing sodium, hydrogen, chloride, nitrate and perrhenate ions.

Perrhenate in solution can be determined gravimetrically as nitron or tetraphenylarsonium perrhenate,[1] but nitrate must be absent in the first of these determinations and interferes in the second unless present in extremely low concentrations.

A number of colorimetric methods for determining perrhenate are available. These include the formation of highly coloured rhenium complexes in reduced solutions with thiocyanate[2] and α-furildioxime,[3] the spectrophotometric determination of hexachlororhenate ion produced by reducing perrhenate in acid solution with hydrazine[4] or chromous chloride[5] and the colorimetric determination of perrhenate with 2:4-diphenylthiosemicarbazide.[6] Rhenium has also been determined spectrophotometrically as tetraphenylarsonium perrhenate in chloroform.[7] Nitrate ion is known to interfere in a number of these determinations.

Custers[8] has determined the absorption spectrum of potassium perrhenate and shown that the ion absorbs strongly in the ultra-violet region of the spectrum. The molar extinction coefficient was 3630 at a wavelength of 230 mμ, and it was indicated that Beer's law was obeyed over the range 221 to 313 mμ for concentrations between 0·02 and 0·0002 *M*.

The absorption of the perrhenate ion in the ultra-violet region does not appear to have been used in the determination of perrhenate, and investigations were therefore undertaken along these lines. The possible use of this method to determine perrhenate in the presence of nitrate was studied.

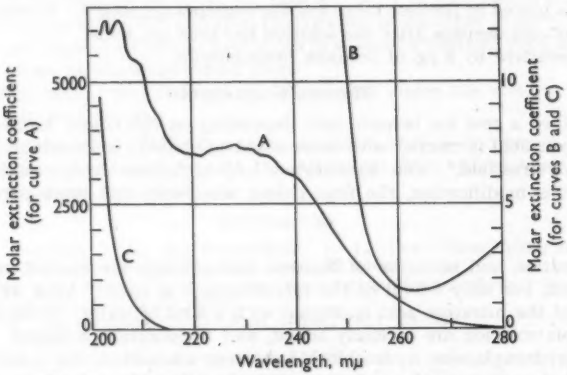


Fig. 1. Absorption spectra: curve A, potassium perrhenate; curve B, potassium nitrate; curve C, potassium chloride

The absorption spectra of the perrhenate, nitrate and chloride ions were determined in calibrated 1-cm quartz cells over the range 202 to 350 mμ, a Unicam SP500 spectrophotometer and solutions of Specpure potassium perrhenate, AnalaR potassium nitrate and AnalaR potassium chloride being used. These absorption spectra are shown in Fig. 1. The absorption spectrum of potassium perrhenate is in close agreement with the results of Custers and of Hindman and Wehner,[9] who used wavelengths longer than 215 mμ. The molar extinction coefficient is 3610 at 228 mμ and 6060 at 206 mμ. The chloride ion starts to absorb appreciably only at wavelengths shorter than 210 mμ, the molar extinction coefficient being 1·0 at 209 mμ. The nitrate ion has an absorption peak at 303 mμ ($\epsilon = 7{\cdot}2$) and a minimum at 264 mμ ($\epsilon = 1{\cdot}6$). There is a sharp rise in the value of the molar extinction coefficient for nitrate as the wavelength is further decreased.

The absorption peak at 228 mμ can be used in the determination of perrhenate in solutions containing no other ions that absorb appreciably at this wavelength. A straight line passing

through the origin is obtained when optical density is plotted against concentration over the range 0 to 50 p.p.m. of rhenium.

The optimum wavelength for the determination of perrhenate in the presence of both nitrate and chloride is 258 mμ. At this wavelength, the molar extinction coefficients for perrhenate and nitrate are 740 and 2·1. At 258 mμ, a straight line is obtained when optical density is plotted against concentration over the range 0 to 250 p.p.m. of rhenium in a 0·65 *M* solution of analytical-reagent grade sodium chloride. (The concentration of sodium chloride in the solutions from a sodium peroxide fusion is 0·65 *M*.) The line does not pass through the origin, but intercepts the optical-density axis at a value of 0·006, which corresponds to the very slight absorption of 0·65 *M* sodium chloride. When the absorption of the sodium chloride is taken into account, it is found that optical-density readings for perrhenate are depressed slightly (2 per cent.) in the presence of 0·65 *M* sodium chloride, but, when analysing solutions of unknown rhenium concentration, allowance for this effect can easily be made by constructing a standard graph, known amounts of rhenium in sodium chloride solution of that concentration being used.

The effect of potassium nitrate on the optical density of a solution containing 125 p.p.m. of rhenium in 0·65 *M* sodium chloride was studied, and the results were in agreement with those calculated from a knowledge of the molar extinction coefficients of nitrate and perrhenate at 258 mμ. In organic rhenium complexes, the ratio of nitrogen to rhenium will rarely exceed 8 to 1. Such a ratio will increase the optical-density reading for perrhenate by less than 2·5 per cent. If necessary, a correction can always be applied for the nitrate in the solution, as nitrogen in organic rhenium complexes can be readily determined by conventional methods of organic analysis. These results show that a method based on the absorption of the perrhenate ion will be satisfactory in the analysis of organic rhenium complexes.

In general, the proposed method, because of its ease of application, should be useful for the rapid determination of perrhenate in simple solutions.

References

1. Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffman, J. I., "Applied Inorganic Analysis," Second Edition, John Wiley & Sons Inc., New York, 1953, p. 320.
2. Geilmann, W., and Bode, H., *Z. anal. Chem.*, 1948, **128**, 489.
3. Meloche, V. W., Martin, R. L., and Webb, W. H., *Anal. Chem.*, 1957, **29**, 527.
4. Meyer, R. J., and Rulfs, C. L., *Ibid.*, 1955, **27**, 1387.
5. Meloche, V. W., and Martin, R. L., *Ibid.*, 1956, **28**, 1671.
6. Geilmann, W., and Neeb, R., *Z. anal. Chem.*, 1956, **151**, 401.
7. Andrew, T. R., and Gentry, C. H. R., *Analyst*, 1957, **82**, 372.
8. Custers, J. F. H., *Physica*, 1937, **4**, 426.
9. Hindman, J. C., and Wehner, P., *J. Amer. Chem. Soc.*, 1953, **75**, 2869.

Chemistry Department
The University, Sheffield

J. B. Headridge
Received *July 4th*, 1958

FLAME-PHOTOMETRIC DETERMINATION OF CALCIUM IN SILICATE ROCKS

In a petrological study of Godolphin granite, Cornwall, one of us (M.S.) wished to study trends in the contents of potassium, sodium and calcium oxides. As the alkalis are determined with a flame photometer, the available literature was examined for a reliable rapid flame-photometric method for small amounts of calcium oxide.

It seemed that, with slight modification, the method described by Edgcombe and Hewett[1] for coke and coal ash could be readily incorporated into the analytical scheme of Shapiro and Brannock.[2] This Note describes the results of investigations into the suitability of this method for silicate rocks of low calcium oxide content.

An E.E.L. flame photometer (Evans Electroselenium Ltd.) was used.

Experimental

The alkali granites from the Godolphin mass near Helston, Cornwall, contain quartz, albite, potash feldspar and white mica, with small amounts of topaz and tourmaline and trace amounts of apatite. They are, therefore, rich in alumina and alkali, but poor in lime and magnesia. Small amounts of titanium, iron, manganese, phosphorus, lithium, boron and fluorine are also present.

Hence, after precipitation of R_2O_3, the only elements likely to interfere are sodium and magnesium. Edgcombe and Hewett have shown that the error caused by sodium oxide is independent of the concentration of calcium oxide and that caused by magnesium oxide varies with the calcium oxide content. They evaluated the following equation—

$$\text{Percentage of calcium oxide (corrected)} = \frac{\text{Percentage of calcium oxide (uncorrected)} \times 100}{100 + (0{\cdot}8 \times \text{percentage of magnesium oxide})} - 0{\cdot}05 \times \text{percentage of sodium oxide.}$$

As the rocks under investigation contain only minor amounts of magnesium oxide (0·2 to 0·4 per cent.), interference from this component is small and can be neglected. Sodium oxide, however, is present in amounts between 4·5 and 6 per cent. (in one instance 10 per cent.), hence it was thought desirable to re-investigate the magnitude of its interference.

Standard solutions were prepared by dissolving anhydrous sodium carbonate and calcium carbonate in 0·5 *N* sulphuric acid. Appropriate aliquots of the standards were transferred to 100-ml calibrated flasks and diluted to volume with 0·5 *N* sulphuric acid from a polythene container.

After turning the sensitivity control clockwise to its fullest extent and setting the instrument to zero against 0·5 *N* sulphuric acid, the scale reading for each solution was recorded. The amounts of standards used and the readings obtained are shown in Table I. The scale readings for six determinations with the same solution are given in the vertical columns A to F in Table I. Before each series, the zero setting was checked.

TABLE I

FLAME-PHOTOMETER SCALE READINGS FOR STANDARD SOLUTIONS OF SODIUM AND CALCIUM OXIDES

Columns A to F represent successive series of determinations on solutions No. 1 to 10. The zero setting was checked before the start of each series, which began with solution No. 1 and ended with solution No. 10

Solution No.	Amount of calcium oxide present, p.p.m.	Amount of sodium oxide present, p.p.m.	Scale reading*						Mean scale reading	Scale reading due to sodium oxide
			A	B	C	D	E	F		
1	5	—	12—	12—	12	12	11·5	12—	11·8	—
2	2·5	—	6	6	6+	6+	6	6	6·1	—
3	5	100	17	17	17	17	16·5	17	16·9	5·1
4	5	100	17—	17	17	17	16·5	17	16·9	5·1
5,	5	50	14·5	14·5	14·5+	14·5	14·5—	14·5	14·5	2·7
6	2·5	100	11	11·5	12	12—	11	11—	11·4	5·3
7	2·5	50	8·5	9	9	9—	8·5	8·5	8·7	2·6
8	—	250	12	12·5	13—	12	12—	12	12·2	12·2
9	—	100	5—	5+	5+	5—	5—	5—	4·9	4·9
10	—	50	2+	2·5+	2·5+	2·5	2+	2·5	2·5	2·5

* + and − indicate that the scale reading was estimated to be one-eighth or a multiple of one-eighth of a scale division (one scale division = 2). In calculating the mean scale readings, + was taken as +0·25 and − as −0·25, *e.g.*, 12− is the same as 11·5+, *i.e.*, 11·75.

The readings are all at the lower end of the scale and correspond approximately to the concentrations of calcium oxide likely to be found in the rocks under investigation. The relationship between scale reading and calcium oxide concentration is linear up to about 25 units on the scale.

The results in Table I confirm Edgcombe and Hewett's statement that the interference from sodium oxide is directly proportional to its concentration (within the errors of calculating the scale reading) and independent of the concentration of calcium oxide. It is apparent that 50 p.p.m of sodium oxide are equivalent to 1 p.p.m. of calcium oxide, *i.e.*, 1 per cent. of sodium oxide increases the result for calcium oxide by 0·02 per cent., not 0·05 per cent. as stated by Edgcombe and Hewett. A correction based on a factor of 0·05 per cent. would give a result for the calcium oxide content of sample No. C_2 (see Table III) much below that calculated from optical measurements.

The results of tests made at higher calcium oxide concentrations were more varied, although the following figures show that the correction factor is still fairly close to 0·02—

Amount of calcium oxide, p.p.m. ..	25	25	10	10
Amount of sodium oxide, p.p.m. ..	100	50	100	50
Factor	0·026	0·018	0·021, 0·017	0·020, 0·014

Method

Reagents—

All reagents are of analytical-reagent grade.

Sulphuric acid, concentrated.

Sulphuric acid, 0·5 N.

Hydrofluoric acid, 40 *per cent. w/v.*

Ammonium hydroxide solution (1 + 1)—Mix equal volumes of ammonium hydroxide solution, sp.gr. 0·880, and water.

Standard calcium solutions—Dry calcium carbonate at 110° C, and prepare solutions containing the equivalent of 10 and 50 p.p.m. of calcium oxide in approximately 0·5 *N* sulphuric acid.

Procedure—

The procedure described is based partly on Edgcombe and Hewett's method[1] and partly on Shapiro and Brannock's method.[2]

Accurately weigh about 0·2 g of powdered rock sample, which has been crushed to pass through a 120-mesh sieve and dried at 110° C, into a platinum crucible. Add 10 ml of hydrofluoric acid and 3 ml of concentrated sulphuric acid. Cover the crucible with a lid and place it on a water bath. Allow the sample to digest overnight or until digestion is complete. Remove the lid and reduce the volume to about 3 ml. (If the method of digestion recommended by Shapiro and Brannock is used, 1 ml of concentrated nitric acid is now added.) Remove the crucible from the water bath and heat gently until fumes of sulphur trioxide are evolved. Allow the crucible and contents to cool, fill the crucible with distilled water and digest on a water bath for about 30 minutes. If there is an insoluble residue, transfer the solution to a Vitreosil beaker, add 20 to 30 ml of distilled water (including washings) and boil for about 10 minutes. (If any residue remains after boiling, filter the solution and examine the residue for calcium-bearing minerals with a petrological microscope.) Cool, transfer the solution to a 200-ml calibrated flask and dilute to the mark with distilled water. Immediately transfer the solution to a polythene container.

By pipette, place a 25-ml aliquot of the solution in a centrifuge tube, add one or two drops of methyl red solution and ammonium hydroxide solution (1 + 1) dropwise until the red colour just changes to yellow. Spin in a centrifuge at about 2500 r.p.m. at 14-cm radius for 3 minutes and then decant the supernatant liquid into a 50-ml calibrated flask. A double precipitation of R_2O_3 is usually necessary. Dilute to the mark with 0·5 *N* sulphuric acid.

Turn the sensitivity control of the flame photometer clockwise to its fullest extent and adjust the zero setting against 0·5 *N* sulphuric acid. Determine the calcium oxide content of the sample solution by comparing the scale reading with that of the standard containing 10 p.p.m. of calcium oxide. If the sample contains more than 10 p.p.m. of calcium oxide, it may be necessary to construct a calibration curve by using the standard solutions containing 10 and 50 p.p.m. of calcium oxide, together with suitable intermediate standards. (In this instance, adjust the sensitivity control to give full-scale deflection with the standard containing 50 p.p.m. of calcium oxide.)

Table II

Determination of calcium oxide in silica brick

Samples 1 to 4 were taken from the same container. Each analyst used a different aliquot of the same sample solution

Sample No.	Amount of calcium oxide found by M. Stone, %	Amount of calcium oxide found by J. E. Thomas, %	Mean amount of calcium oxide found, %
1	1·71	1·66	1·69
2	1·75	1·67	1·71
3	1·67	1·69	1·68
4	1·66	1·68	1·67
		Average ..	1·69

Results

To test the proposed procedure for silicate rocks, determinations were made on four samples from one bottle of British Chemical Standard No. 267 (silica brick). Two 25-ml aliquots of each sample solution were analysed. One set of samples was analysed by M.S., the calcium oxide

content being determined from the mean of twenty instrument readings, and the other set by J.E.T., the calcium oxide content being determined from the mean of thirteen instrument readings. The results, which are shown in Table II, are lower than the average of the nine analyses given with the sample (1·75 per cent.), but are well within the range stated (1·66 to 1·80 per cent.), and, in fact, show a slightly greater precision than the gravimetric and volumetric determinations.

In addition, determinations were made on four samples, each taken from separate bands in the banded "granite" sheets near Porthleven, Cornwall. The results, which are shown in Table III, are in good agreement with determinations made by using ethylenediaminetetra-acetic acid. However, it should be noted that the second decimal place is stated for comparison in these Tables only; it has no real significance in the method used.

TABLE III

AMOUNTS OF CALCIUM AND SODIUM OXIDES IN TOPAZ - TOURMALINE - ALBITE GRANITES

Sample No.	Amount of sodium oxide found, %	Amount of calcium oxide found (uncorrected), %	Amount of calcium oxide found (corrected), %	Amount of calcium oxide found by titration with ethylenediamine-tetra-acetic acid, %
B_1	5·51	0·47	0·36	0·32
B_2	4·32	1·13	1·04	1·02
C_1	4·43	0·47	0·38	0·41
C_2	5·46	0·35	0·24	0·23

CONCLUSIONS

The proposed procedure can be applied to rocks containing up to about 2·5 per cent. of calcium oxide. It is sufficiently accurate for calculating normative values and compares favourably with existing rapid methods. Provided that a calibration curve is used, it is possible to determine amounts of calcium oxide up to about 7·5 per cent. The correction factor for sodium oxide is more variable, hence results are liable to be less accurate for rocks containing appreciable amounts of this component.

The time taken for a single analysis (including five scale-reading observations), once the rock has been digested and the sample solution diluted to volume, is about 30 minutes.

REFERENCES

1. Edgcombe, L. J., and Hewett, D. R., *Analyst*, 1954, **79**, 755.
2. Shapiro, L., and Brannock, W. W., "Rapid Analysis of Silicate Rocks," *U.S. Geol. Survey Bull. No.* 1036C, 1956.

DEPARTMENT OF GEOLOGY
UNIVERSITY COLLEGE
KEELE, STAFFORDSHIRE

M. STONE
J. E. THOMAS
Received *May 8th*, 1958

THE DETERMINATION OF NITROGEN IN CERTAIN FLUORINATED COMPOUNDS BY THE KJELDAHL METHOD

PUBLISHED methods for the determination of nitrogen in fluorinated organic compounds have been reviewed by Macdonald.[1] The only reference to an attempt to use the Kjeldahl method appeared in a paper by Rush, Cruickshank and Rhodes,[2] who stated that, for perfluoro compounds, the method was usually unsuccessful.

We publish this Note in order that this almost complete absence of reference to the Kjeldahl method shall not give the impression that it cannot be used for the analysis of any fluorinated materials.

We have successfully analysed a variety of solid fluorinated materials, some typical results being shown in Table I. The procedure of Belcher and Godbert[3] was followed, except that we distilled into standard hydrochloric acid instead of boric acid, as previous experience with non-fluorinated materials had shown that more consistent results would be obtained in this way.

It can be seen from Table I that no interference was caused by the presence of fluorine, even in the analysis of compounds containing nitro groups, which were subjected to phosphorus - hydriodic acid reduction[3] before digestion.

TABLE I

DETERMINATION OF NITROGEN IN FLUORINATED COMPOUNDS

Compound	Calculated amount of nitrogen, %	Amount of nitrogen found, %	Calculated amount of fluorine, %
$C_6H_5{\cdot}NH{\cdot}CO{\cdot}CF_3$	7·41	7·36, 7·36, 7·45, 7·37, 7·40, 7·39	30·14
$CF_3[CH_2{\cdot}O{\cdot}CO{\cdot}C_6H_4{\cdot}NO_2]_2$ ..	6·83	6·64, 6·73	9·26
$CH_3{\cdot}CO{\cdot}NH{\cdot}C_6H_3(CF_3){\cdot}NO_2$ ..	11·29	11·23, 11·21	22·97
$C_6H_5{\cdot}CO{\cdot}CF_2{\cdot}C(:NOH){\cdot}C_6H_5$ ..	5·09	4·83, 4·85	13·80
$[\cdot(CF_3)_2{\cdot}CO_2H{\cdot}C_6H_3{\cdot}NH_2]_2$ ..	5·88	5·90, 5·93	31·91
$[\cdot CF_2{\cdot}CO{\cdot}O{\cdot}NH_4]_2$*	12·50	12·02, 12·03	33·91
$C_3F_7{\cdot}CH_2{\cdot}O{\cdot}P(O){\cdot}[NH{\cdot}C_6H_5]_2$ ..	6·51	6.45, 6·36	30·91
$[C_3F_7{\cdot}CH_2{\cdot}O]_2P(O){\cdot}NH{\cdot}C_6H_5$ ..	2·61	2·61, 2·60	49·52

* Analysis for other constituents indicated that this compound was not quite pure.

As might be expected, some slight etching of the digestion flasks takes place, but this is, in fact, an advantage in that it markedly reduces bumping of the digestion mixture, which is otherwise liable to occur.

We thank Mrs. M. W. Roberts for experimental work and Mr. E. J. P. Fear and Dr. I. M. White for supplying samples.

REFERENCES

1. Macdonald, A. M. G., *Ind. Chem. Mfr.*, 1957, **33**, 360.
2. Rush, C. A., Cruickshank, S. S., and Rhodes, E. J. H., *Mikrochim. Acta*, 1956, 858.
3. Belcher, R., and Godbert, A. L., "Semi-Micro Quantitative Organic Analysis," Second Edition, Longmans, Green & Co. Ltd., London, 1954, p. 102.

ROYAL AIRCRAFT ESTABLISHMENT
FARNBOROUGH, HANTS.

T. R. F. W. FENNELL
J. R. WEBB
Received *May 8th*, 1958

A NEW pH INDICATOR

A NEW pH indicator has been prepared by condensing sodium 1:2-naphthaquinone-4-sulphonate with 2:4-dinitrophenylhydrazine.

The mechanism of the reaction is analogous to the condensation of asymmetric alkyl- and arylphenylhydrazines with 1:2-naphthaquinone, and the product is an *o*-hydroxyazo compound.[1]

The advantages of the new indicator are (*a*) it can be easily prepared in a pure crystalline state, (*b*) it is soluble in water, ethanol and ethanol - diethyl ether mixtures, (*c*) it changes colour over a narrower pH range than most other indicators, (*d*) small concentrations of it are effective, (*e*) it is equally effective either in titrating acid with alkali or *vice versa*, (*f*) it is extremely sensitive, even when titrating 0·001 *N* solutions, (*g*) ageing, particularly with respect to air and light, has no effect on it, and (*h*) it is suitable for preparing indicator test-papers.

EXPERIMENTAL

PREPARATION OF THE INDICATOR—

A solution was prepared by dissolving 0·99 g (0·005 mole) of 2:4-dinitrophenylhydrazine in 100 ml of hot ethanol. While still hot, this solution was gradually added, with continuous shaking, to a solution of 1·3 g (0·005 mole) of sodium 1:2-naphthaquinone-4-sulphonate in 25 ml of hot distilled water. The reaction mixture, which assumed a dark red colour, was then boiled for 10 minutes. A blood-red crystalline solid separated from the hot solution. After 30 minutes, this was removed by filtration and recrystallised from glacial acetic acid.

The yield of recrystallised product was 1·7 g, which was found to contain 43·22 per cent. of carbon, 2·53 per cent. of hydrogen, 12·21 per cent. of nitrogen, 6·98 per cent. of sulphur and 4·7 per cent. of sodium. The formula $C_{16}H_9O_6N_4SNa$ requires 43·64 per cent. of carbon, 2·05 per cent. of hydrogen, 12·73 per cent. of nitrogen, 7·27 per cent. of sulphur and 5·2 per cent. of sodium.

A stock solution was prepared by dissolving 0·1 g of solid indicator in 125 ml of 96 per cent. ethanol and diluting to 250 ml with distilled water. This solution was orange-red; it was stored in a brown bottle.

pH RANGE—

Buffer solutions with pH values between 5·2 and 12 were prepared. To 10 ml of each buffer solution, 3 drops of indicator solution were added. The colour was rose-red in buffer solutions of pH below 8·4, and violet at pH values above 9·2. In the pH range 8·4 to 9·2, the indicator produced purple-violet colours.

INDICATOR TEST-PAPERS—

A Whatman No. 1 filter-paper was cut into small strips, soaked in a 0·1 per cent. solution of the indicator in 50 per cent. ethanol for 15 minutes and then dried. The test-paper assumed a rose-red colour and was sufficiently sensitive to detect 0·01 *N* solutions of alkalis.

APPLICATIONS OF THE INDICATOR

The indicator has been used in determining the acidity of urine and vinegar, the acid value of oils and water-insoluble organic acids, and also in the urease test. It is not suitable for titrating free sulphuric acid in a solution of copper sulphate or for assaying vegetable alkaloids. For example it forms crimson crystals with strychnine, a purple-violet colour with brucine and an intense blue colour with atropine. This can be used to differentiate between strychnine and brucine if a few crystals of each are treated with 2 drops of indicator solution on a white porcelain plate.

REFERENCE

1. Friez-David, H. E., Blangey, L., and Kaul, H., *Helv. Chim. Acta*, 1946, **29**, 1765.

BIOCHEMISTRY DEPARTMENT
FACULTY OF VETERINARY MEDICINE
CAIRO UNIVERSITY
GIZA, CAIRO

M. Z. BARAKAT
S. K. SHEHAB
M. M. EL-SADR

Received *July 15th*, 1958

REDOXOKINETIC TITRATION—A NEW ELECTROANALYTICAL TECHNIQUE

Doss and Agarwal[1] discovered the rectifying property of reversible electrodes, which they designated the "redoxokinetic effect," since the rectification is dependent on the kinetics of oxidation - reduction reactions at the electrodes. They elaborated[2] the theory of the effect, their deductions being based on the theory of absolute reaction rates proposed by Glasstone, Laidler and

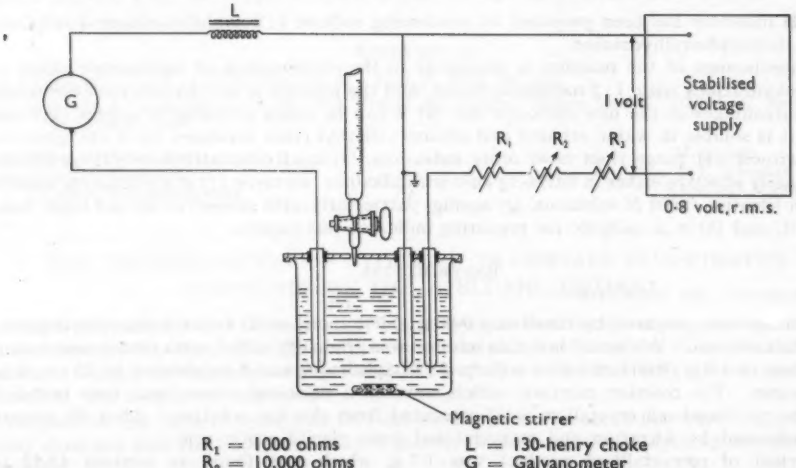


Fig. 1. Arrangement for redoxokinetic titration

Eyring.[3] A more general theory has recently been formulated by Barker, who, in addition, evolved a new electroanalytical technique known as radio-frequency polarography.[4] The application of

the effect to the determination of end-points in redox titrations is described in this Note. The arrangement of apparatus is shown in Fig. 1.

Acidified ammonium ferrous sulphate solution was titrated against potassium permanganate solution. After each addition of permanganate, the redoxokinetic potential was determined at a fixed a.c. potential. In normal redoxokinetic work, a small a.c. voltage (10 mV, r.m.s.) is generally used, as this helps in the quantitative interpretation of results. For this work, however, it is advantageous to use higher voltages (25 to 100 mV, r.m.s.). A typical set of results for the titration of 25 ml of 0·02 *N* ammonium ferrous sulphate (acidified with 100 ml of 4 *N* sulphuric acid) with approximately 0·02 *N* potassium permanganate is shown in Fig. 2, the visual end-point being indicated by an arrow. The apparent surface area of the electrode was 0·1 sq. cm, and the a.c. voltage was 50 mV, r.m.s. It has been found that the end-point, which is accompanied by the greatest change in redoxokinetic potential, can be determined with an error of $\pm 0{\cdot}4$ per cent. at a concentration of 0·02 *N*, which shows the suitability of the technique for analytical work.

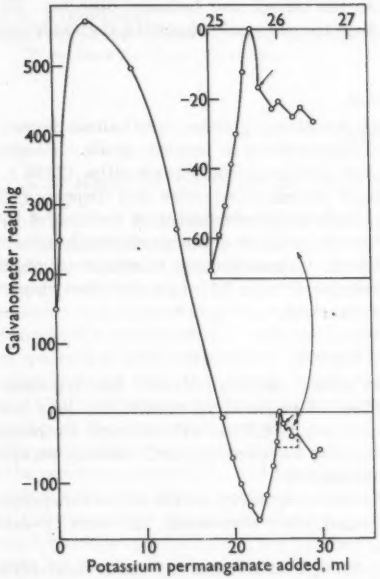


Fig. 2. Redoxokinetic titration of 25 ml of 0·02 *N* ammonium ferrous sulphate with approximately 0·02 *N* potassium permanganate at constant a.c. potential

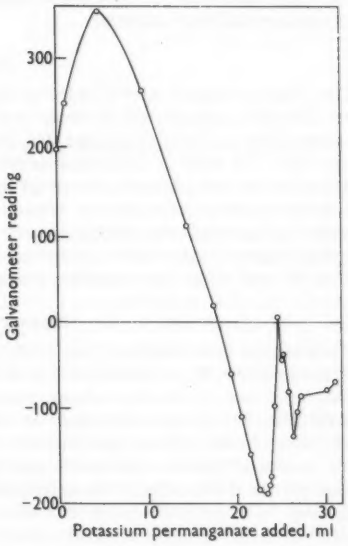


Fig. 3. Redoxokinetic titration of 25 ml of 0·02 *N* ammonium ferrous sulphate with approximately 0·02 *N* potassium permanganate under constant current conditions

A redoxokinetic titration can be carried out under constant current conditions, *i.e.*, without re-adjustment of the a.c. potential to a particular value after each addition of reagent. The galvanometer is read after each addition. A typical set of results for the titration of 25 ml of 0·02 *N* ammonium ferrous sulphate (acidified with 100 ml of 4 *N* sulphuric acid) with approximately 0·02 *N* potassium permanganate at a constant current of 200 μA, r.m.s., is shown in Fig. 3. The apparent surface area of the electrode was 0·1 sq. cm. This modification simplifies the procedure, and the end-point can be determined with the same precision as before.

It is interesting to note that the end-point often coincides with a galvanometer reading of almost zero, but it must be pointed out that, by adjusting the composition of the solution to be titrated, it is possible for the galvanometer reading to be widely different from zero at the end-point. It is hoped that the significance of this, and the general theory of redoxokinetic titration, will be dealt with in a separate paper.

References

1. Doss, K. S. G., and Agarwal, H. P., *J. Sci. Ind. Res.*, 1950, **9B**, 280.
2. ——, ——, *Proc. Indian Acad. Sci.*, 1951, **34**, 263; 1952, **35**, 45.
3. Glasstone, S., Laidler, K. J., and Eyring, H., "The Theory of Rate Processes," McGraw-Hill Book Co. Inc., New York, 1941, p. 575.
4. Barker, G. C., *Anal. Chim. Acta*, 1958, **18**, 118.

Central Electrochemical Research Institute
Ramanad District
Karaikudi, India

K. S. G. Doss
U. H. Narayanan
K. Sundararajan

Received *June 11th*, 1958

IDENTIFICATION OF GLASS FRAGMENTS BY THEIR PHYSICAL PROPERTIES

The measurement of the physical properties of glass fragments, particularly density and refractive index, is commonly used in forensic problems as a means of identifying the object from which the fragments originated.[1 to 5] The reliability with which identification can be made depends on the variation of these physical properties, both within the object and between objects. To find the amount of variation, measurements have been made of the physical properties of glass fragments from sixty-one different objects.

Experimental

The objects studied were bottles of several types, headlamp glasses, ophthalmic lenses and window and plate glasses, all of which are commonly encountered in forensic work. Fragments from these objects were examined for fluorescence at two exciting wavelengths (2536 A and between 3000 and 4000 A), and measurements were made of refractive index and dispersion (both determined with an Abbé refractometer) and density, both by displacement of water and by the use of sensitive density columns.[6] These columns were prepared in a thermostatically controlled glass tube by successively adding twenty-six bromoform - bromobenzene mixtures of regularly decreasing density. After the column had been set aside for at least 72 hours, the glass chips were placed in it, and their distribution at equilibrium was noted.

Results and discussion

Fluorescence was observed only with ophthalmic lenses, although Marris[7] has reported the use of this property in the examination of window glass. One hundred ophthalmic lens blanks, including a number of bifocals, were examined, only one of which did not exhibit some fluorescence. In general, different colours were observed at the two exciting wavelengths, and, without exception, the two parts of each bifocal lens showed different fluorescence.

The results of dispersion measurements offered little encouragement for the use of this property. When significant differences between fragments were found, they were small, and were invariably accompanied by large differences in refractive index.

Absolute measurements of refractive index on thirteen ophthalmic lenses from several different sources gave results between 1·5230 and 1·5242 ($\pm$0·0001), which suggests that ophthalmic lenses cannot readily be differentiated one from another by this property, although, as a group, they have a considerably higher refractive index than the other glasses. Bottles also tended to group together; the refractive indexes of thirty-one widely different types of bottle were between 1·5120 and 1·5170. However, headlamps and window and plate glasses showed wide variations in refractive index. Observations of relative refractive index were also made by examining the Becke lines of chips of glass in a refracting medium. It was found that the refractive-index range of the immersion liquid over which the Becke line disappeared depended partly on the orientation of the chip on the microscope stage and partly on the edge observed. The reliability of these observations was improved by using a universal stage and selecting a chip edge for which the range was a minimum. Some objects were sampled at several different positions, and, in all instances, variation in refractive index from point to point was found to be negligible.

Densities of fragments were measured to an accuracy of $\pm$0·00004 g per cc at 20° C, and a scatter diagram of density against refractive index revealed some degree of correlation between these properties; this has also been observed by Gamble, Burd and Kirk.[2] The densities of the ophthalmic lenses were in a restricted range (2·5084 to 2·5184), but those of the other types of object showed wide ranges. Density differences from point to point in a single bottle were found to be quite large, which agrees with the findings of Sawai, Tashiro and Umeya.[8] This was

studied further by examining the distributions of a number of chips from the same bottle in density-gradient columns. Such studies showed wide spreads of density within bottles. The largest spread observed was estimated to be 0·00230 g per cc, and the total range of density of thirty of the thirty-one bottles studied was 0·0225 g per cc. Other randomly selected bottles showed spreads estimated as 0·00194, 0·00133, 0·00075 and 0·00030 g per cc. The other types of object showed much smaller spreads, which can probably be neglected.

References

1. Kirk, P. L., "Crime Investigation. Physical Evidence, and Police Laboratory," Interscience Publishers Inc., New York, 1953.
2. Gamble, L., Burd, D. Q., and Kirk, P. L., *J. Crim. Law Criminol.*, 1943, **33**, 416.
3. Roche, G. W., and Kirk, P. L., *Ibid.*, 1947, **38**, 168.
4. Greene, R. S., and Burd, D. Q., *Ibid.*, 1949, **40**, 85.
5. Kirk, P. L., and Dollar, A. M., *Ibid.*, 1949, **39**, 684.
6. Kirk, P. L., "Density and Refractive Index. Their Application in Criminal Identification," Charles C. Thomas, Springfield, Ill., 1951, p. 49.
7. Marris, N. A., *Analyst*, 1934, **59**, 686.
8. Sawai, I., Tashiro, H., and Umeya, K., *Bull. Inst. Chem. Res., Kyoto Univ.*, 1952, **30**, 40.

Dominion Laboratory
Department of Scientific and Industrial Research
Wellington, New Zealand

J. Finch
P. P. Williams

First received *December 23rd*, 1957
Amended, *August 1st*, 1958

Apparatus

A MODIFIED DESIGN OF THE AUDUS SOIL-PERFUSION APPARATUS

Lees and Quastel[1] described an apparatus in which soil was continuously perfused with a dilute solution of ammonium sulphate, the rate of nitrification of the ammonia being followed by quantitative nitrate determinations on the perfusate. The perfusate was circulated by positive air pressure, which was produced with an alternating water pump. The apparatus was somewhat cumbersome, and Audus[2] modified the design so that negative pressure was used to lift the perfusate to the top of the soil column. This less bulky design was used successfully in this department for several years for soil nitrification studies.[3] Temple[4] modified the Audus design to a smaller form consisting of only two parts, which, however, required some adjustment before perfusion began. The apparatus was rather complicated and was more difficult to construct than either the Audus or the proposed perfuser.

The enrichment technique for the isolation of various soil bacteria was greatly simplified by using the perfusion apparatus, and Lees[5] claimed to have isolated pure cultures of nitrifying bacteria on a column of glass beads perfused with dilute solutions of ammonia. Soil perfusion was used in this laboratory for the enrichment of soil samples before the attempted isolation of nitrifying bacteria from soils in which very few of these organisms were present. The use of large numbers of perfusers led to the development of a more compact design, which, however, still used the same principle as the Audus apparatus. The small size of the apparatus permitted a large number of perfusers to be housed in a small incubator.

Description of the apparatus

The perfuser (see Fig. 1) was made from a 500-ml Pyrex-glass Florence flask, A, the neck of which was removed and replaced by the tube D (15 cm long and 2·5 cm internal diameter). The soil sample was introduced at the top of tube D, the rubber stopper being immediately replaced. At the point of fusion into the flask, tube D was constricted to 0·5 cm to prevent soil from falling into the flask. A constriction of diameter 1 to 2 mm was made in the side-arm, C, at its point of fusion, B, into the flask, and the side-arm was then bent at 90° and extended to the same height as the top of flask A. For smooth operation of the apparatus, the constriction at B had to be less than 2 mm in diameter so as to supply the necessary resistance to impede the flow of liquid back into the flask. Without this resistance, air was drawn into the flask instead of up tube F with the perfusate. Tube F had an internal diameter of 7 mm, and was fused into the right-angle bend of the side-arm, C. This position was empirically found to give the best flow of air and perfusate up tube F, the other end of which was fused into tube D about 5 cm from the top. Tube E, which led to a water pump, had the same internal diameter as F and was fused into the top of tube D and into flask A. The air flow was controlled by a clip on the rubber tubing leading

to the pump. Tube E joined tube D about 2 cm above tube F to prevent loss of perfusate by suction into tube E. No constriction was necessary in tube E. A cotton-wool plug protected the open end of tube E from contamination, and a piece of glass tubing, G, filled with cotton-wool, was placed in the top of the side-arm C to reduce the air flow into tube F and to prevent aerial contamination of the perfusate.

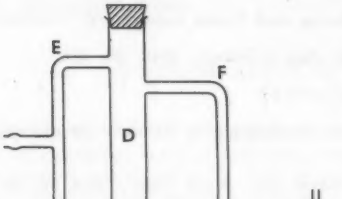


Fig. 1. Modified soil perfuser

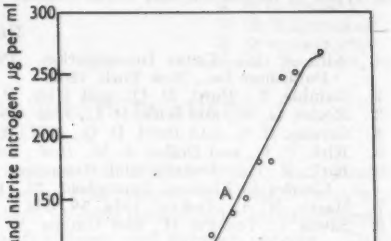


Fig. 2. Accumulation of nitrate and nitrite nitrogen on perfusion of 30 g of garden soil with 300 ml of 0·01 *M* ammonium sulphate for 21 days at 30° C: curve A, nitrate nitrogen; curve B, nitrite nitrogen

Method of operation

Fresh garden soil collected from the 0 to 2-inch horizon was air-dried for 24 hours and then thoroughly mixed with 0·3 per cent. of a soil conditioner (Krilium, obtained from Monsanto Chemicals, Australia). The 0·5 to 2·0-mm crumb fraction was collected. Thirty grams of soil were then carefully introduced into tube D, layers of glass-wool being placed above and below the soil column to hold the crumbs in place and prevent "puddling." The soil was perfused with 300 ml of 0·01 *M* ammonium sulphate for 4 weeks in an air-jacketed incubator thermostatically controlled at 30° ± 2° C. The initial level of liquid in the reservoir was marked on the side of flask A and was kept constant by adding sterile distilled water at least 2 hours before the daily sample was collected for analysis. The level of liquid in A was adjusted so that the flask was at least half full, and 300 ml of perfusate was found to be most satisfactory. When the level of liquid fell below halfway, the perfusate did not ascend tube F and perfusion became irregular. The nitrification rate in the soil was determined by plotting the nitrate-N content of the perfusate against time. When the perfusion experiment had been completed, the soil sample was shaken from tube D, and the apparatus was thoroughly washed in hot soapy water and cleaned with acid to remove all traces of carbonate and soil. The perfuser was then washed several times with distilled water and finally with water containing 0·2 per cent. of sodium hydrogen carbonate. The open ends of the perfuser were plugged with cotton-wool and covered with paper to prevent saturation of the plugs with water. The perfusers were sterilised by heating in an autoclave at 120° C for 20 minutes.

Results

When the nitrate-N in the perfusate was plotted against time, a sigmoid type of curve similar to that reported by Lees and Quastel[1] was obtained. Typical results are shown in Fig. 2. No nitrate or nitrite formation was detectable for 2 days, after which nitrite formation increased markedly. The rate of formation was linear until a maximum of 100 µg of nitrite-N per ml was reached after incubation for 12 days. After 10 days, the nitrite was utilised at an increasing rate, and could no longer be detected in the perfusate by the eighteenth day. Nitrate was detected after 2 days, and its rate of formation then increased rapidly and became linear by the ninth day.

The nitrate-N increased to 250 μg per ml after incubation for 18 days, and reached a peak of 270 μg per ml by 21 days; ammonia could no longer be detected after the twelfth day. The perfusate initially contained 280 μg of ammonia-N per ml, almost all of which was later detected as nitrate-N.

Perfusion of both soil crumbs and glass-bead columns with dilute ammonia solutions showed that the proposed apparatus worked satisfactorily, both qualitative and quantitative tests showing that, as the ammonia disappeared, it was replaced by nitrite and later nitrate.

Discussion of results

The apparatus described has been used in this laboratory, together with the Audus perfuser, for quantitative nitrification studies on soil for 12 months. It was also used to detect very small numbers of nitrifiers in desert soils from Central Australia, and also for the partial purification of nitrifiers by Lees's method.[5] However, pure cultures of nitrifying bacteria were not obtained after prolonged perfusion in either the Audus or the proposed apparatus. In all experiments, the nitrification curves closely resembled those reported by Lees and Quastel,[1] *i.e.*, the alteration to the design of the perfuser did not affect the growth or the nitrifying activity of the ammonia-oxidising bacteria. The only difference noted when results obtained with the proposed apparatus were compared with the earlier reports was the transient accumulation of extensive amounts of nitrite-N in the perfusate before nitrate was detected. The nitrite accumulation appeared to be a characteristic of the apparatus, as it was observed in all quantitative experiments.

The perfuser had several advantages over earlier designs. It was smaller and more compact, and stood on the bench without the support of a retort stand. More than twenty perfusers were operated by a single water pump through a manifold fitted in an incubator 4 feet × 3 feet × 2 feet. The pump was run slowly, as only slight negative pressure was sufficient to lift the perfusate to the top of the soil column. The noise from the water pump was overcome, a desirable factor when the perfusers were in a room constantly used by other workers.

The proposed perfuser was made in one piece, which overcame the problem of breakages that occasionally occurred when the Audus apparatus was assembled or dismantled. In spite of its compact design, the apparatus could be cleaned quickly and easily. Finally, the apparatus was easy to construct and relatively cheap to produce. The design would be useful in all problems for which the Audus perfuser was suitable, and its small size rendered it particularly valuable when large numbers of perfusers had to be housed in a limited space.

We thank Mr. H. Uffelmann for technical assistance in the manufacture of the perfusers.

References

1. Lees, H., and Quastel, J. H., *Biochem. J.*, 1946, **40**, 803.
2. Audus, L. J., *Nature*, 1946, **158**, 419.
3. Collins, F. M., *Austral. J. Agric. Res.*, 1954, **5**, 688.
4. Temple, K. L., *Soil Sci.*, 1951, **71**, 209.
5. Lees, H., *Nature*, 1951, **167**, 355.

Department of Bacteriology
University of Adelaide
South Australia

C. M. Sims
F. M. Collins

Received *June 17th*, 1958

Book Reviews

THE UFAW HANDBOOK ON THE CARE AND MANAGEMENT OF LABORATORY ANIMALS. Edited by ALASTAIR N. WORDEN, M.A., B.Sc., M.R.C.V.S., F.R.I.C., and W. LANE-PETTER, M.A., M.B., B.Chir. Second Edition. Pp. xx + 951. London: The Universities Federation for Animal Welfare. 1957. Price 70s.

Every year, in the United Kingdom alone, something between two and a half and three million animals are used in laboratories for standardising pharmaceutical and pesticidal preparations, including some of the "pure" active principles of which they are formulations, for research in the medical and paramedical fields, as well as into general biological problems, and for teaching purposes, mainly to medical students, but also to those wishing to qualify in physiology, pharmacology, biochemistry and some other specialised disciplines of biological science. For all these purposes it is of the essence of the contract that the animals shall be alive at the time of the experiment; their use is subject to the provisions of the 1876 Cruelty to Animals Act, as administered by the Home Office and its specially appointed and highly qualified inspectors.

The uses of these animals are manifestly pretty varied; the procedures and techniques that these uses elicit are even more varied than might consequently have been expected. They include simple feeding tests involving changes in dietary composition so subtle that nothing but experiments on animals can reveal them or, alternatively, with so direct a bearing on the health of animals that only experiments on animals can be hoped to provide an answer to the posited problem; single or serial injections of substances intended to protect against specific infections, generally bacterial or viral; operations for the partial or total removal of a particular gland or tissue, with a view to studying the deprived animal's reactions to stimuli of normal or abnormal kinds and to establishing the relation of the gland or organ to normal physiological processes or their pathological disturbances; and experiments on whole animals subjected to extremes of temperature, pressure or humidity, conducted for the light they may throw on the increasingly complex range of conditions, including radiation hazards, to which *Homo sapiens*—and to a less extent his domestic and farmyard animals themselves—is being exposed.

Anyone who holds either that these goals are none of them worth aiming at, or the commoner and equally unrealistic view that their importance is insufficient to justify the use of sentient but inevitably non-consenting animals, will not want to read, and even more certainly will not want to buy, the much enlarged new edition of the UFAW Handbook, already highly esteemed by all those who have to breed or use laboratory animals; what is more, he will not need to read this review and is advised to stop at this point, if he has already gone so far. For this is not the place, if there is one, in which to argue with those who think (or say they think) that experiments on animals are incapable of giving answers to problems of human health and disease and who sometimes even go so far as to extend these alleged doubts to animal health and disease; nor would it be fitting to enter into ethical and philosophical arguments with those who, prepared to admit that useful knowledge can be gained by these means, deny that it is permissible for moral man to do so.

Rather is it my intention here to emphasise another fact that should have a special appeal to the scientifically objective observer, whether or not he is directly himself involved in the problems of laboratory animal husbandry. This book, of which the first edition appeared over 10 years ago and was under half the size of the second, gives overt evidence of a meeting-point for two parties only too often thought by superficial or biased observers to be for ever irreconcilable. The humanitarian—who might in this context more suitably be dubbed the animalitarian or even the philozoist—is clearly concerned to ensure that unnecessary suffering is not undergone by any animal, outside or inside a cage; he generally goes further than this and takes a more positive attitude, insisting on the animal's "right" to be as contented as it is possible for man, with or without the active connivance of nature, to make him. Thus it is not a matter for suprise that a body such as The Universities Federation for Animal Welfare should be taking the problems of the laboratory animal with the utmost seriousness. In this book there are, besides chapters on more general matters, such as the legal aspects of the situation, equipment of all kinds (from water bottles and hypodermic needles to cages and ventilation systems), nutrition, breeding, record keeping, choice of species and strain, pests and hygiene, also individual chapters on some thirty-four species of laboratory mammals belonging to the rodent, lagomorph, insectivore and carnivore groups, four chapters on the domesticated ungulates, two on primates, one on marsupials and four on birds, not to mention some chapters on the cold-blooded vertebrates and several on invertebrates. There is also a chapter of notes on species not elsewhere dealt with in the book: this constitutes

in itself a guide to a somewhat esoteric zoo, its inhabitants ranging from raccoons, bobcats, lemmings and susliks, through vampire bats, locusts and tse-tse flies, to leeches and the snail, *Lymnaea*; *Drosophila*, needless to say, has already had a chapter to itself.

From this it seems so clear as to make its mention a work of supererogation that the Federation, when in its title it refers to animal welfare, means the welfare of all animals, however obscure and to some even unpleasant. Except, it may seem, when the animal may fairly be described as a "pest" towards one of the more warmly regarded "higher" species, though the Federation would presumably press for humane methods of exterminating even lice and bed-bugs.

Before such rather extreme problems of kind-heartedness arise, there will have taken place the somewhat unexpected meeting to which I have already alluded. The experimental or analytical user of animals had, before and during the first years of World War II, been getting increasingly anxious about his supplies of laboratory animals. Not only was he finding it more and more difficult to get enough of them, and particularly of certain species, but their somewhat vaguely defined and assessed "quality" was, if anything, deteriorating. In his own interests, both scientific, for animals in a poor condition will as often as not provide the experimenter and analyst with equivocal results, and economic, because bought animals that die before you can use them are of less than no interest to anyone—even to the vendor who has already been paid for them—he was compelled to examine in detail the conditions under which his experimental animals were being produced and the effect of those conditions on the animals' quality.

The results of this examination were already known to some, and had been even assiduously brought by a few of them to their colleagues' attention, though they were expected by many and welcomed by all. The efficiency of any laboratory animal as a "tool" in standardisation or investigation was directly correlated with its bodily and "mental" health; put in another way, the number of animals required in any test or experiment to provide significance of results at any pre-determined level bears an inverse relationship to the well-being of the animal. You could either get better results with the same number of healthier animals or equally trustworthy results with a smaller number of healthier animals. Thus science and economics on the one hand found common cause with charity on the other. Even if there had ever been the slightest justification for believing that scientists, and especially those working in fundamental or applied biology, were less scrupulous in their behaviour or less sincere in their kindliness towards "lower animals" than any otherwise similar group of men and women—and there never has been any evidence at all for so disquieting a view—it would now have been clear that it pays to be kind to "our dumb (and not so dumb) friends."

The permanent alliance to which this book is eloquent witness will no doubt be a thorn in the flesh of those who, from good motives and ignorance or from evil motives and dishonesty, have insisted that the animal lover and the "vivisectionist" are poles asunder. On the contrary, there is a territory, the territory covered by this book, over which their interests are not merely compatible, but complementary and often identical.

And so it is that this book must be permanently in the hands of everyone who is responsible for the provenance, whether by purchase or breeding, of laboratory animals or their use as scientific reagents. It will help him to correct any errors he may be—as who is not occasionally?—already committing with species of animals currently in use; it will be an essential standby against the day, which will almost certainly come, when he is called on to handle animals of a species previously alien to his laboratory. Indeed, it may well help him to decide whether it will be well to add to the species available to him, and if so which and how.

Thus it is by no means only the laboratory animals themselves that will benefit from a wide circulation of this comprehensive and authoritative volume. Among them the laboratory mouse must presumably be reckoned the first, at any rate on the basis of counting paws; nearly three-quarters of the animals used in British laboratories are mice. But when the advantages to different species are "weighted" to take account of other factors than mere numbers, probably the guinea-pig will turn out to be the chief gainer. The state of the pre-war laboratory guinea-pig had to be seen often to be believed—and for the results it furnished to be disbelieved. The improvement is already enormous, and this is in no small measure due to the activities of the Laboratory Animals Centre (formerly the Laboratory Animals Bureau), one of the direct outcomes of the searching examination carried out during the war years into current conditions for producing and distributing laboratory animals. No doubt the combined efforts of the Bureau and UFAW will secure further continuous improvements to the benefit of animal and user alike; the joint editorship of this book, indeed, ensures that these improvements will be both morally acceptable and scientifically sound.

A. L. BACHARACH

CHROMATOGRAPHIC TECHNIQUES: CLINICAL AND BIOCHEMICAL APPLICATIONS. Edited by IVOR SMITH, B.Sc., Ph.D., F.R.I.C. Pp. xiv + 309. London: William Heinemann Medical Books Ltd. 1958. Price 45s.

Many volumes on chromatography have now appeared in print, and interest in the applications of this technique is becoming wider each day. This may be partly because with this procedure we are enabled to identify microgram amounts of complex materials without any prior separation. It is also because chromatographic technique is relatively simple and, to some extent, artistic, and, once the procedure has become familiarised, there is a constant challenge to apply the ideas to any new problems that arise. But it is probably in the biological sciences that most use is made of chromatography; it is with this field that Dr. Smith's book deals mostly. Nevertheless, it is, in general, an excellent concise exposition of the present position in regard to the technique used in chromatography. At the same time, the key references are present for those who wish to delve more deeply into the fundamentals of the procedure. In the main, the book deals with the applications of paper chromatography; it is actually a compilation of chromatographic applications to various biochemical subjects, and each chapter has a specialist author. The introductory chapters by Dr. Smith discuss the type of apparatus recommended in the book, its development and its limitations. The general principles of paper chromatography are outlined, mainly from the practical angle. These include such subjects as drop size, type of solvent, line of flow, location of agents and R_F values, described in a fashion easy for the reader to comprehend. A description of de-salting and related technique is a further contribution by Dr. Smith. The importance of de-salting in the chromatography of organic compounds is fully discussed, together with the various techniques and apparatus used. Electrolytic de-salting, ion-exchange methods of analysis and solvent extraction are given consideration. The chapter on separation and identification of amino acids constitutes the most important part of the book. Types of solvents used, Tables of R_F values for most amino acids with various solvents and the specific-location reagents for the chemical groupings in the amino acids make this chapter most valuable for reference by the practical worker. Here also are shown some of the abnormal chromatograms produced in certain pathological conditions wherein amino acid metabolism is disturbed. A chapter on the chromatography of indoles and imidazoles contains useful Tables of R_F values and also photographs of many two-way chromatograms on the subject. Purines and pyrimidines are also given full treatment, but it seems that the chapter on sugars is unnecessarily condensed. Keto-acids, phenolic acids and those in the citric cycle have more recently been the subject of chromatographic survey, and this work is well described. The chapter on barbiturates is of much interest to workers in the forensic field; Mr. Jackson of the Metropolitan Police Laboratory has contributed this section.

The complexity of steroid chemistry and the need for research into the adrenal hormones has led to a search for new techniques, which have largely been based on chromatography. Some outline of this work is given by Mr. Edwards. The final chapters are on methods used for the investigation of new problems and also some model experiments for students. The biochemist will certainly find this book most helpful and workers in other fields, *e.g.*, agriculture, may also profit from extending the applications given therein. R. F. MILTON

MICRODIFFUSION ANALYSIS AND VOLUMETRIC ERROR. By EDWARD J. CONWAY, M.D., D.Sc., F.R.I.C., F.R.C.P.I., F.R.S. Fourth Edition. Pp. xviii + 465. London: Crosby Lockwood & Son Ltd. 1957. Price 42s.

In common with other scientific fields, analytical chemistry continues to expand at an ever-increasing rate, and there is a steady trend towards the application of small-scale methods of analysis in all branches of analytical chemistry. As a result of this, there has been a notable expansion of chemical literature during the last decade, and textbooks become virtually "out-dated" almost as soon as they are published. The task of the writer is consequently made more difficult in keeping abreast with developments, and textbooks are becoming more in the nature of reviews than accounts of the writers' own experiences and appreciations, which are of greater value.

This cannot be said of the book under review, which has retained its character of a treatise of the practical experiences of Professor Conway, now universally accepted by virtue of its previous editions. The technique of microdiffusion is an important tool that, although intended originally for application in the biochemical sphere, has many other uses when small-scale (milligram or

microgram scale) methods of analysis are essential. In the same way, Professor Conway's treatment of volumetric error is of value to both the student and the analyst.

In this latest edition, Professor Conway has extended the text to include many new methods and applications that have been advanced during the past decade and also describes new developments in apparatus. For example, an up-to-date treatment of blood analysis includes the blood ammonium method; a description of methods for total nitrogen, glutamine and glutamic acid; diffusion methods for determination of cyanide, sulphide, phenols, methanol and *iso*propanol and volatile poisons of toxicological interest. Methods for determining enzymes, such as monoamine oxidase, and of histaminase and acetylcholinesterase have also been included. The determination of formaldehydogenic steroids and of glycine by application of the well known chromotropic acid reaction with formaldehyde is described, glycine being determined by its reaction with ninhydrin to yield formaldehyde, which is separated by diffusion. Also of interest is the use of the diffusion technique for the determination of acetaldehyde, which is absorbed by semicarbazide, the technique being used to determine indirectly lactic acid in blood and tissues. An up-to-date treatment of halogen determinations, including their determination in organic material, is given in the text, together with recent methods for determination of carbon monoxide.

Improvements in the design of the standard microdiffusion units and components are described.

Although this book is written generally for the biochemist, it is obviously one of great value to all analysts, since many of the methods described can be applied to a wide range of materials, for which reason the book should be accessible in every laboratory. G. INGRAM

MANUAL OF ANALYTICAL METHODS RECOMMENDED FOR SAMPLING AND ANALYSIS OF ATMOSPHERIC CONTAMINANTS. By the Committee on Recommended Analytical Methods, American Conference of Government Industrial Hygienists. Loose leaf, viii + 50 pages (11 methods). Cincinnatti, Ohio: American Conference of Government Industrial Hygienists, 1958. Price $5.00.

This Manual of eleven methods for the trace determination of ten toxic substances occurring in industrial atmospheres is the outcome of 13 years' work by a series of committees, which have included a number of members well known in this specialised field of analysis. The work has been sponsored by the American Conference of Government Industrial Hygienists, and is complementary to the toxicological surveys undertaken by this organisation, which lead to the annual publication of lists of threshold limits or maximal allowable concentrations.

The Manual aims to provide industrial hygienists with approved procedures for the sampling and analysis of atmospheric contaminants. Minimal requirements have been established for approval; methods should be sufficiently sensitive to measure one-tenth of the threshold limit, the air-sampling rate and sample volume should not exceed specified values, and the precision should be such that the average deviation obtained by at least ten collaborating analytical laboratories is not greater than 10 per cent. This collaborative confirmation is one of the most notable features of this Manual, but it is not extended to cover the air-sampling procedure. Simplicity has not been a major consideration, and, unlike the well known methods issued originally by the Department of Scientific and Industrial Research and now continued by the Factory Department of the Ministry of Labour, the procedures require skilled personnel and considerable laboratory facilities.

The chemical reactions used in these methods are well established, with the exception of that for arsenic, in which arsine produces a red colour with silver diethyldithiocarbamate in pyridine. Lead and mercury are determined by dithizone methods, and chlorinated hydrocarbons by reaction with sodium to liberate chloride ion, and then argentimetric titration. Other methods included in the Manual are for hydrogen sulphide (by titration), manganese, oxides of nitrogen (phenoldisulphonic acid) and parathion; there is both a colorimetric and a polarographic method for sulphur dioxide. It is rather surprising that a titration method has been selected for formaldehyde when several good colorimetric procedures are available.

The methods are clearly presented, and the accuracy, sensitivity and interferences are listed. It is to be hoped that some of the more common contaminants of industrial atmospheres, which are missing from the present Manual, will be the subject of the same systematic study.

J. C. GAGE

KUNSTSTOFF-, LACK- UND GUMMI-ANALYSE: CHEMISCHE UND INFRAROTSPEKTROSKOPICHE METHODEN. By Dr. rer. nat. DIETER HUMMEL. (Two Volumes.) Text Volume: Pp. 409; Volume of Plates: 183 plates (548 spectra). Munich: Carl Hanser Verlag. 1958. Price DM.148.00; 259s.

This book, which deals with the analysis of synthetic polymers, natural resins and gums, is written from a rather novel angle. Although the older chemical methods, which have long been used in attempted characterisations of these substances, are described, the bias in this book is always towards the infra-red structure.

The book is in two parts. In Part I, detailed methods of preparation of samples for infra-red test are given. Then the various resins and polymers are described, and very few natural or synthetic polymers are omitted, *e.g.*, copal resin and cellulose as well as polythene and polytetrafluoroethylene all find a place. In addition, the first part includes information about plasticisers, stabilisers and aids to vulcanisation.

The second part gives practical examples of the infra-red spectra of the various natural resins, synthetic polymers and plasticisers that are described in the first part.

There are several points of criticism. For example, (1) the author index to the first part is extremely unsatisfactory. Many of the names of joint authors of papers are omitted, and reference back from the index to the text of the book cannot be made without prolonged search. (2) In Part II, it is desirable that the spectra of proprietary preparations should be reduced to a minimum and particularly so when the chemical composition of the synthetic resin is not given. (3) It is unlikely that the binding of Part II, which contains the spectra, will stand up to the wear and tear of the laboratory bench.

Nevertheless, the analyst in the plastics and allied industries will do well to provide himself with copies of these two parts, and particularly so if he believes, as he should, that infra-red spectra of natural and synthetic resins will supplement his chemical work. J. HASLAM

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Edited by J.-A. GAUTIER. Quatrième Serie: Pp. vi + 209; Cinquième Serie: Pp. iv + 161; Sixième Serie: Pp. iv + 171. Paris: Masson et Cie. 1956; 1957; 1958. Price 2400 fr.; 2500 fr.; 2600 fr.

A further three issues, Nos. 4, 5 and 6, have appeared under this title since the last review. The general style and arrangements are similar to those of the first three issues.

Two contributions are notable for their comprehensive survey of their author's chosen field and extensive classified bibliography. These are an article on foreign substances in foods (in No. 4) and one on the examination of fruit juices (in No. 5). The fourth issue also contains competent articles on recent advances in chromatography of carbohydrates, the quantitative acetylation of hydroxyl groups and on methods of dealing with samples of perishable and unstable foodstuffs, with special reference to milk. On the other hand, a brief account of coulometric titrations (in No. 5) with no bibliography is of little value to anyone wishing to pursue this subject further.

The analysis of wines forms the subject of two articles. In No. 4, the determination of total solids is dealt with at length from both theoretical and practical standpoints. Drying *in vacuo* at 70° C is the favoured procedure. The biological testing for preservatives in wines is described in No. 5. One would conclude from the opening paragraph of this article that the author has strong feelings on this matter, but the statement that chemical and physical methods are always more sensitive, more precise and more specific than biological methods must be regarded as a far too sweeping assertion.

In the 6th issue, Professor Duval describes the extensive thermogravimetric studies made by his school using the Chevenard thermobalance. The apparatus and technique are described in detail, and the results are summarised. Also worthy of note in this issue is an article on the analytical use of sodium tetraphenylboron and one on the techniques involved in the use of radioactive elements in chemical analysis.

Several articles could be criticised on grounds of omission or of superficial treatment. Two examples must suffice. Gas - liquid chromatography is briefly described in an article on the application of physico-chemical methods to the analysis of fats, but without any reference to the original papers of James and Martin. The short account of the use of the thermal-conductivity cell (katharometer) as a detector in gas chromatography is only likely to mislead those who are

unfamiliar with this instrument. The published work of de Whalley and his co-workers is ignored in an article that purports to deal with the use of ion exchange and chromatography in the analysis of the products of the sugar refinery.

The general impression remaining after reading these reviews is one of wide variation in the value of individual contributions. P. MORRIES

SCIENTIFIC GLASSBLOWING. By E. L. WHEELER. Pp. xxii + 478. New York and London: Interscience Publishers Inc. 1958. Price $9.75; 75s.

In the preface, the author states that his aim is to provide not only a text-book for the beginner in glass blowing, but also a manual that will enable a glassblower to serve as a general laboratory technician or even to "make of himself the research chemist's co-worker." This explains why rather less than half the content of this book is concerned with the manipulation of glass, but does not excuse the poor choice of title. The instruction in glassworking is pedestrian and within the scope of existing literature on the subject. Only some of the important characteristic properties of glass are mentioned, and these in cursory fashion. The bearing that these factors have on problems of annealing, strain, fracture, thermal shock and reliability of components is not explained at all. Likewise, the strain-viewer is described, but the interpretation of strain-patterns is not attempted, even in simple terms. In short, this section of the book lacks the knowledge without which the student glassworker cannot master his medium. The treatment of this aspect of the subject is so weak that one doubts if the author himself has acquired the necessary understanding.

The remainder of the book comprises chapters on the deposition of metals, purification of mercury, fractional distillation, high vacuum, metal working, electric heaters and miscellaneous equipment and procedure. Here is assembled a mass of information based largely on manufacturers' brochures and prior publications. Whether or not a trained glassworker should concern himself with these matters is debatable. His proper function is to visualise the scientist's needs and employ his skill in translating them into the reality of useful glass apparatus. Done well, this alone will secure him an honourable place in the research team.

The editing and proof-reading do not measure up to the quality of book-production. Twelve pages are made available for the contents list, and room has been found for a very large number of illustrations. However, many corrections are called for. Disregarding simple errors in spelling and grammar, attention is directed to the following. Chapter VIII refers to Housekeeper's work on copper seals. His name and date of publication are misquoted, and Fig. 5B on p. 168 misinterprets his instructions. It is necessary to refer to an original paper to understand the function of the apparatus drawn incorrectly in Fig. 6 on p. 252. In the expression for pumping speed (p. 341) it should not be left to the reader to guess most of the units of measurement. At the end of Chapter XVI, Tables V and VI each have several errors, Table VII has one and Table VIII is too empirical to be considered seriously. C. H. SIMMS

Publications Received

TABLES OF INTERATOMIC DISTANCES AND CONFIGURATION IN MOLECULES AND IONS. Scientific Editor: L. E. SUTTON, M.A., D.Phil., F.R.S. Special Publication No. 11. Pp. 391. London: The Chemical Society. 1958. Price 42s.; $6.00.

WATER TREATMENT. By G. F. MUGELE, B.Sc. (Eng.), A.M.I.C.E., A.M.I.W.E., and A. WISEMAN, B.Sc., A.R.I.C. Pp. x + 141. London: George Newnes Ltd. 1958. Price 21s.

SYMPOSIUM ON SPECTROCHEMICAL ANALYSIS FOR TRACE ELEMENTS. Papers presented at the Sixtieth Annual Meeting of the American Society for Testing Materials, Atlantic City, N.J., June 18th, 1957. Pp. vi + 79. Philadelphia, Pa.: American Society for Testing Materials. 1958. Price $2.75.
ASTM Special Technical Publication No. 221.

THE STRENGTHS OF CHEMICAL BONDS. By T. L. COTTRELL. Second Edition. Pp. x + 317. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1958. Price 32s.; $7.00.

AUTOMATIC MEASUREMENT OF QUALITY IN PROCESS PLANTS. Proceedings of a Conference held at Swansea, September 23rd to 26th, 1957, by the Society of Instrument Technology. Pp. xii + 320. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1958. Price 50s.; $9.50.

THE PERIODIC TABLE. By D. G. COOPER, B.Sc., F.R.I.C. Pp. x + 86. London: Butterworths Scientific Publications. 1958. Price 6s. 6d.

CHROMATOGRAPHIE VON STERINEN, STEROIDEN UND VERWANDTEN VERBINDUNGEN. By R. NEHER. Pp. viii + 100. Amsterdam, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd. 1958. Price 16s.

QUANTITATIVE ORGANISCHE MIKROANALYSE (PREGL - ROTH). Seventh Edition. By Dr. H. ROTH. Pp. xiv + 361. Vienna: Springer-Verlag. 1958. Price 81s. 6d.; $11.85.

SPECIFICATIONS AND METHODS OF ANALYSIS FOR CERTAIN PESTICIDES. Compiled at the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries and Food. *Technical Bulletin No.* 1. Third Edition. Pp. x + 126. London: Her Majesty's Stationery Office. 1958. Price 7s.

BIOLOGICAL LABORATORY DATA. By L. J. HALE, Ph.D. Pp. x + 132. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1958. Price 15s.

NOMENCLATURE OF ORGANIC CHEMISTRY. Rules issued by certain Commissions of the International Union of Pure and Applied Chemistry. Pp. vi + 92. London: Butterworths Scientific Publications. 1958. Price 15s.

This volume contains Definitive Rules for Section A: Hydrocarbons, and Section B: Fundamental Heterocyclic Systems, issued by the Commisssion on the Nomenclature of Organic Chemistry of I.U.P.A.C. ("The I.U.P.A.C. 1957 Rules"), *Definitive Rules for Nomenclature of Steroids issued by the Commissions on the Nomenclature of Organic Chemistry and the Nomenclature of Biological Chemistry of the I.U.P.A.C.* ("The I.U.P.A.C. 1957 Rules for Nomenclature of Steroids"), *and Tentative Rules for Nomenclature in the Vitamin B_{12} Field recommended by the Commission on the Nomenclature of Organic Chemistry of the I.U.P.A.C.*

EXERCISES IN THE EVALUATION OF DRUGS AND SURGICAL DRESSINGS. By E. J. SHELLARD, B.Pharm., F.P.S., A.R.I.C., F.L.S. Pp. xviii + 158. London: Pitman Medical Publishing Co. Ltd. 1958. Price 15s.

THE DETERMINATION OF NITROGEN IN STEEL. A Report of the British Iron and Steel Research Association. Pp. xii + 146. London: The Iron and Steel Institute. 1958. Price 37s. 6d. *Iron and Steel Institute Special Report No.* 62.

ELECTROANALYTICAL CHEMISTRY. By JAMES J. LINGANE, B.Chem., Ph.D. Second Edition. Pp. xiv + 669. New York and London: Interscience Publishers Inc. 1958. Price $14.50; 109s.

CALENDAR OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN 1958–1959. Pp. vi + 306. London: The Pharmaceutical Press. 1958. Price 20s.

AN INTRODUCTION TO PATHOLOGY. By G. PAYLING WRIGHT, D.M., F.R.C.P. Third Edition. Pp. xii + 660. London, New York and Toronto: Longmans, Green & Co. Ltd. 1958. Price 45s.

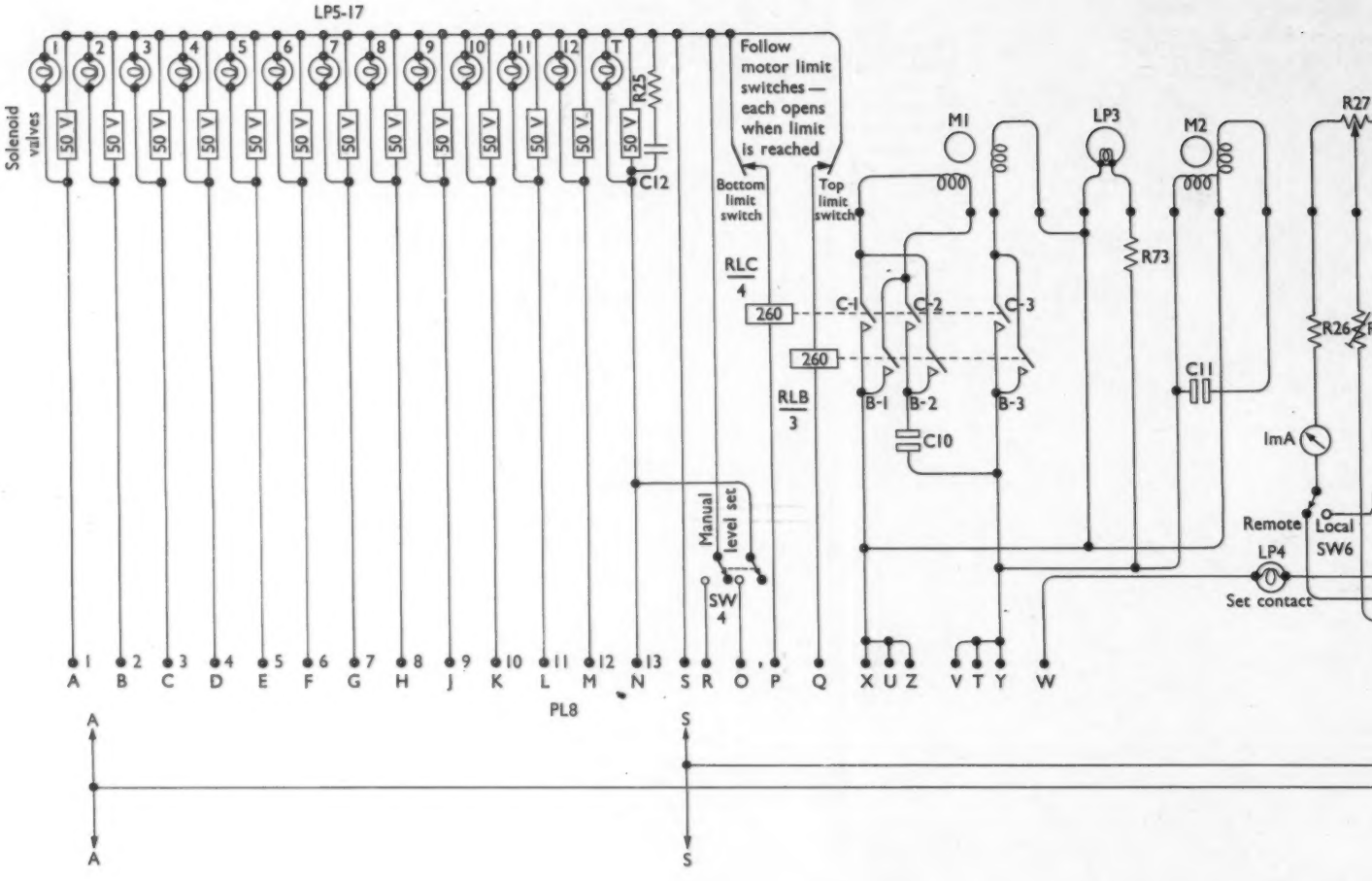
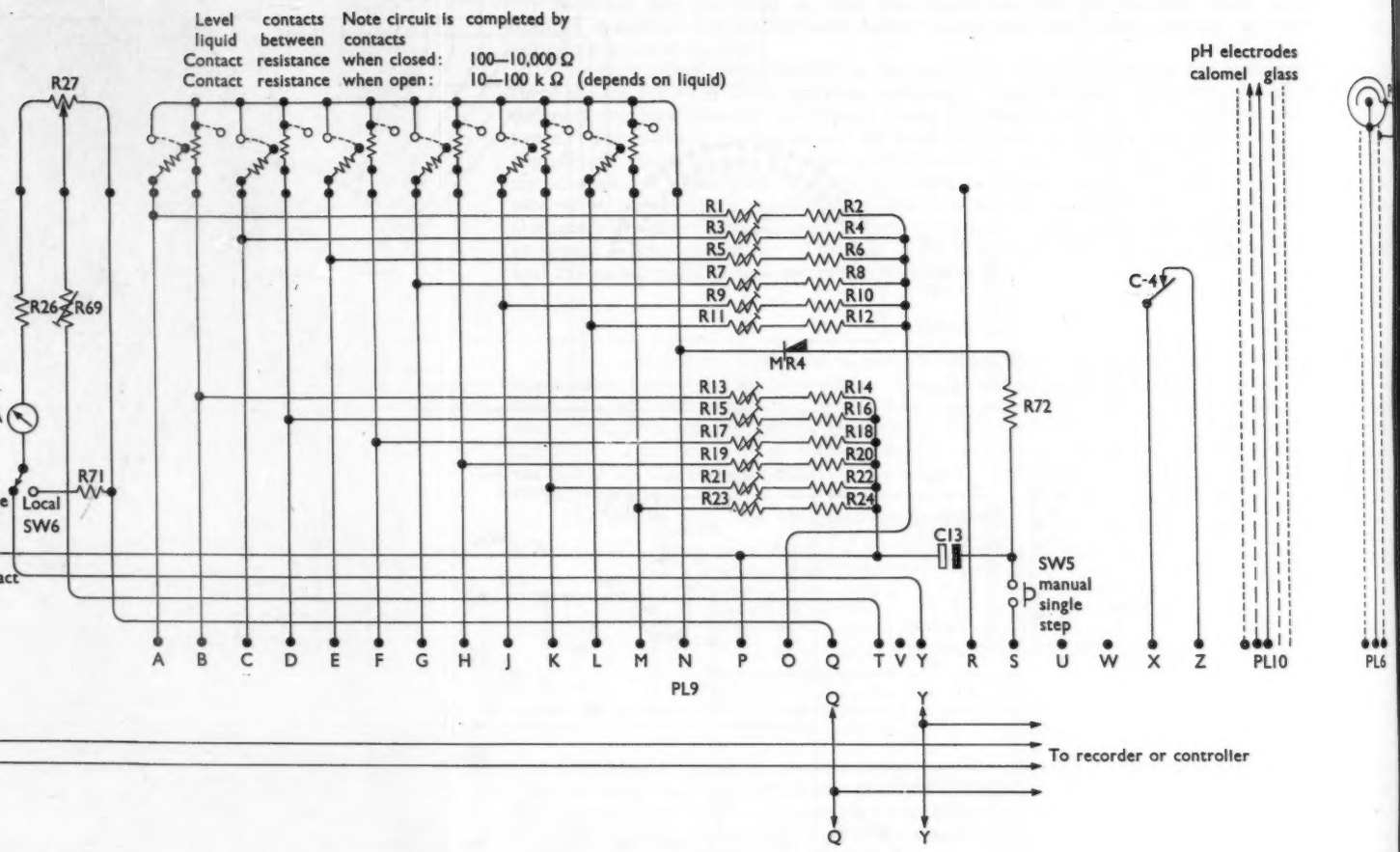


Fig. 9. Circuit diagram of distribution unit (fo



n unit (for values of components, see Appendix, p. 502)